

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
22 November 2001 (22.11.2001)

PCT

(10) International Publication Number  
**WO 01/88121 A1**

(51) International Patent Classification<sup>7</sup>: **C12N 15/10,**  
15/63, 15/70, 1/21

Geert [BE/BE]; Pontstraat 16, B-9820 Merelbeke (BE).  
RENARD, Jean-Pierre [BE/BE]; Peter Benoitlaan 141,  
B-9050 Gentbrugge (BE). BOGAERT, Thierry [BE/BE];  
Wolvendreef 26G, B-8500 Kortrijk (BE).

(21) International Application Number: **PCT/IB01/01068**

(74) Agents: BALDOCK, Sharon, Claire et al.; Boult Wade  
Tenant, Verulam Gardens, 70 Gray's Inn Road, London  
WC1X 8BT (GB).

(22) International Filing Date: 18 May 2001 (18.05.2001)

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,  
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,  
ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0012233.3 19 May 2000 (19.05.2000) GB

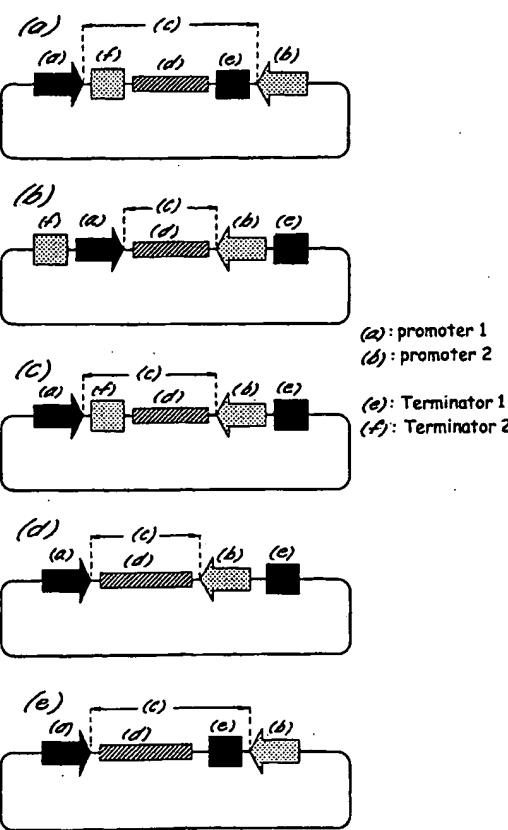
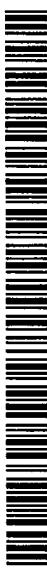
(71) Applicant (for all designated States except US): DEV-  
GEN NV [BE/BE]; Technologiepark 9, B-9052 Zwij-  
naarde (BE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): PLAETINCK,

[Continued on next page]

(54) Title: VECTOR CONSTRUCTS



(57) Abstract: Vector constructs useful in the expression of double-stranded RNA. The constructs are particularly useful for expression of double-stranded RNA in vitro and in vivo.

**WO 01/88121 A1**



(84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG)

**Declaration under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU,*

**Published:**

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

- 1 -

### VECTOR CONSTRUCTS

#### Field of the invention

The invention relates to improved vector constructs for use in the expression of double-stranded RNA, particularly for use in the expression of double-stranded RNA *in vitro* and *in vivo*.

#### Background to the invention

Since the advent of double-stranded RNA inhibition (RNAi) as a tool for controlling gene expression, as described in WO 99/32619 and WO 00/01846, there has been recognised a need for specialised vectors designed for the production of double-stranded RNA (dsRNA).

Cloning vectors designed to produce high levels of dsRNA have been previously described by Plaetinck et al. (WO 00/01846) and Timmons et al. *Nature*, 395:854 (1998). These vectors generally contain a multiple cloning site (MCS) into which target DNA fragments can be cloned flanked by two opposable transcriptional promoters. Essentially, these three components (Promoter 1, MCS and Promoter 2) make up the entire system. In the appropriate expression system, the DNA cloned into the MCS may be transcribed in both directions, leading to the production of two complementary RNA strands.

A disadvantage of the known systems is that not only the cloned fragment is transcribed. Read-through of the RNA polymerase will result in transcription of the entire vector, and this also in both directions. As only transcription of the cloned DNA fragment will result in active dsRNA for RNAi purposes, transcription of the vector part results in useless,

- 2 -

inefficient RNA. More specifically, 80% of these transcripts can be considered as non-specific and thus non-effective.

The large amounts of non-specific RNA generated by the prior art plasmid and expression systems results in some undesirable side effects. First, in RNAi protocols based on introduction of dsRNA into *C. elegans* via a food organism such as *E. coli* which expresses the dsRNA (see WO 00/01846), large RNA strands are considered to be toxic for the food organism. As a result, high amounts of RNA accumulating in *E. coli* cause a significant part of the population to die. Second, and probably more important, is the reduction of inhibition potential. The presence of large amounts of non-specific dsRNA causes a competitive environment for the specified sequences. The potential of the template-specified dsRNA sequences to inhibit the targeted protein expression in, for instance, *C. elegans* cells is reduced by the presence of these large non-specific regions. Such an inhibition by non-specific dsRNA has also been shown in *Drosophila* by Tushl et al., Genes & Development 13:3191-3197 (1999). Not only the potential to inhibit gene expression is affected, but also the amount of specific dsRNA produced is limited. Third, transcription of the vector backbone part, more particularly transcription of the origin of replication and related structures, results in plasmid instability and plasmid reorganisation, leading to reduced production of dsRNA. This relatively low concentration of effective dsRNA in turn leads to inefficient RNAi.

To conclude, the previously described vectors have following shortcomings: they are toxic to the

- 3 -

feeding organism, a greater proportion of the transcripts produced are non-specific, the inhibitory potential of the dsRNA is reduced by the presence of non-specific regions, a high incidence of plasmid 5 reorganizations and loss of plasmid from the feeding organism. It is therefore an object of the present invention to provide improved vectors for the production of dsRNA which avoid the disadvantages of the prior art vectors.

10 Vectors for use in the *in vitro* synthesis of RNA transcripts, for example the production of RNA probes, have been known and commonly used in the art for some time (see for example F. M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994); Jendrisak et al, Vectors for *in vitro* production of RNA copies of either strand of a cloned DNA sequence, US 4,766072). In standard *in vitro* transcription protocols the problem of read-through transcription of vector sequences is generally 15 avoided by linearizing the transcription vector at restriction site positioned at the 3' end of the desired transcript. However, this solution is not appropriate for *in vivo* transcription or for the production of dsRNA where it is important that the 20 template is transcribed in both directions.

25 The inventors now propose a novel solution to the problems encountered with the prior art vectors for the production of dsRNA, based on the use of transcription terminators. Generally the solution 30 consists of the use of at least one transcription terminator operably linked to at least one promoter, wherein the terminator stops the transcription initiated by the promoter. Any DNA fragment inserted between the 3' end of the promoter and the 5' end of

- 4 -

the terminator will then be transcribed, without the unwanted transcription of the vector backbone.

Preferentially the vector consists of two promoters and two terminators, as further described below.

5 Therefore, in accordance with a first aspect of the invention there is provided a DNA construct comprising two opposable promoters flanking an inter-promoter region, the construct further comprising at least one transcription terminator positioned  
10 transcriptionally downstream of one of the said promoters In particular, the invention provides for:  
a DNA construct comprising:

- a) a first promoter and
- b) a second promoter,

15 in which the first and second promoter are in opposite orientation to each other and define:

c) an inter-promoter region positioned downstream of the 3' end of the first promoter and downstream of the 3' end of the second promoter;

20 and which DNA construct further comprises:

d) at least one cloning site positioned in the inter-promoter region; and

e) a first transcription terminator, positioned (as seen from the 3' end of the first promoter)

25 downstream of the first promoter and downstream of the at least one cloning site, wherein the first transcription terminator is operably linked to the first promoter.

The inter-promoter region can also further be  
30 defined as: the DNA region between the 3' end of the first promoter and the 3' end of the second promoter, and which is downstream of the first promoter, and which is downstream of the second promoter, and which preferably does not contain the 5' end of the first

- 5 -

promoter and of the second promoter. The opposable first promoter and second promoter drive expression directional from their 5' ends to their 3' ends starting transcription downstream of their 3' ends, 5 thus providing transcription of both strands of any nucleotide sequence(s) present in the inter-promoter region.

The two promoters present in the DNA construct of the invention may be identical or they may be 10 different and may be of essentially any type. The precise nature of the promoters used in the construct may be dependent on the nature of the expression system in which the construct is expected to function (e.g. prokaryotic vs eukaryotic host cell).  
15 Bacteriophage promoters, for example the T7, T3 and SP6 promoters, are preferred for use in the constructs of the invention, since they provide advantages of high level transcription which is dependent only on binding of the appropriate RNA polymerase. Each of 20 these promoters can independently be chosen. The phage promoters can also function in a wide variety of host systems, i.e. both prokaryotic and eukaryotic hosts, provided that the cognate polymerase is present in the host cell.  
25 The arrangement of two "opposable" promoters flanking an inter-promoter region such that transcription initiation driven by one of the promoters results in transcription of the sense strand of the inter-promoter region and transcription 30 initiation driven by the other promoter results in transcription of the antisense strand of the inter-promoter region is an arrangement well known in the art, for example, in the pGEM7 series of vectors from Promega Corp., Madison WI, USA.

- 6 -

The DNA constructs of the invention differ from those of the prior art because of the presence of at least one transcription terminator positioned transcriptionally downstream of one of the promoters.

5 The transcription terminator may be uni- or bi-directional, the choice of uni- vs bi-directional terminators being influenced by the positioning of the terminator(s) within or outside the inter-promoter region, as explained below. The terminator may be of  
10 prokaryotic, eukaryotic or phage origin.

Bacteriophage terminators, for example T7, T3 and SP6 terminators, are particularly preferred. The only requirement is that the terminator must be capable of causing termination of transcription initiating at the  
15 promoter relative to which it is transcriptionally downstream. In practice, these means that the promoter and terminator must form a 'functional combination', i.e. the terminator must be functional for the type of RNA polymerase initiating at the  
20 promoter. By way of example, a eukaryotic RNA pol II promoter and a eukaryotic RNA pol II terminator would generally form a functional combination. The selection of a functional combination is particularly important where bacteriophage promoters and  
25 terminators are to be used in the constructs of the invention, since the phage promoters and terminators are both polymerase-specific. To form a functional combination both the promoter and the terminator should be specific for the same polymerase, e.g. T7  
30 promoter and T7 terminator, T3 promoter and T3 terminator etc.

In one embodiment, the DNA construct of the invention may comprise a single transcription terminator, positioned (as seen from the 3' end of the

- 7 -

first promoter) downstream of the first promoter and downstream of the at least one cloning site, wherein the first transcription terminator is operably linked to the first promoter, wherein the single  
5 transcription terminator is positioned in the inter-promoter region

In an alternative arrangement, the DNA construct comprises a single transcription terminator positioned outside of the inter-promoter region. In a still  
10 further embodiment, the DNA construct may comprise two transcription terminators, each one of which is positioned transcriptionally downstream of one of the two promoters. In this arrangement, one or both of the terminators may be positioned within the inter-  
15 promoter region. These various embodiments of the DNA constructs of the invention will be more fully described below, with reference to the accompanying drawings. The position of a first transcription terminator outside the inter-promoter region may also  
20 be further defined as, i.e. such that a first transcription terminator is positioned (as seen from the 3' end of the first promoter) downstream of the first promoter, downstream of the at least one cloning site, and downstream of the 5' end of the second  
25 promoter.

The position of a second transcription terminator outside the inter-promoter region may also be further defined as, i.e. such that a second transcription terminator positioned (as seen from the 3' end of the second promoter) downstream of the second promoter, downstream of the at least one cloning site, and downstream of the 5' end of the first promoter.  
30

Moreover, when the terminator is not located in the inter-promoter region, the distance between the 5'

- 8 -

end of the first promoter and 3' end of the second terminator, or the distance between the 5' end of the second promoter and the 3' end of the first terminator is preferably small, i.e. such that the 3' end of the  
5 first transcription terminator is separated from the 5' end of the second promoter by no more than 2000 nucleotides, preferably no more than 1000 nucleotides, more preferably no more than 500 nucleotides, even more preferably no more than 200 nucleotides,  
10 especially preferably no more than 100 nucleotides, more especially preferable no more than 50 nucleotides, even more especially preferably no more than 20 nucleotides, particularly preferably no more than 10 nucleotides, more particularly preferably no  
15 more than 6 nucleotides.

Furthermore, when the second transcription terminator is located outside of the inter-promoter region, preferably the 3' end of the second transcription terminator is separated from the 5' end  
20 of the first promoter by no more than 2000 nucleotides, preferably no more than 1000 nucleotides, more preferably no more than 500 nucleotides, even more preferably no more than 200 nucleotides, especially preferably no more than 100 nucleotides,  
25 more especially preferably no more than 50 nucleotides, even more especially preferably no more than 20 nucleotides, particularly preferably no more than 10 nucleotides, more particularly preferably no more than 6 nucleotides

30 As defined above the term 'inter-promoter region' refers to all of the DNA sequence between the two promoters. As explained above, in certain embodiments of the invention the transcription terminator(s) may be sited within the inter-promoter region. The inter-

- 9 -

promoter region may, advantageously, comprise a sequence of nucleotides forming a template for dsRNA production. The precise length and nature of this sequence is not material to the invention. The 5 invention further provides DNA constructs in which the inter-promoter region comprises a cloning site. The function of the cloning site is to facilitate insertion of a DNA fragment forming a template for dsRNA production between the two promoters. Thus, the 10 invention provides a series of cloning vectors which are of general use in the construction of template vectors for dsRNA production. Also encompassed within the scope of the invention are vectors derived from the cloning vectors which have a DNA fragment inserted 15 into the cloning site.

The cloning site may further comprise one or more of the following:

- at least one restriction site, (as known in the art), or one or more further restriction sites, 20 e.g. to provide a multiple cloning site(as known in the art),
- a stuffer DNA, e.g., flanked by at least two restriction site, such as two *BstXI* restriction sites, or two *XcmI* restriction sites,
- 25 - *attR1* and *attR2* recombination sites,
- a *ccdB* nucleotide sequence,
- a *ccdB* nucleotide further comprising at least one unique blunt-end restriction site, such as a *SrfI* restriction site, and/or
- 30 - a DNA fragment inserted in the at least one cloning site.All of the DNA constructs provided by the invention may, advantageously, form part of a replicable cloning vector, such as, for example, a plasmid vector. In addition to the opposable

- 10 -

promoters, inter-promoter region and transcription terminator(s), the vector 'backbone' may further contain one or more of the general features commonly found in replicable vectors, for example an origin of replication to allow autonomous replication within a host cell and a selective marker, such as an antibiotic resistance gene. The selective marker gene (e.g. the antibiotic resistance gene) may itself contain a promoter and a transcription terminator and it is to be understood that these are completely independent of the promoter and terminator elements required by the invention and are not to be taken into consideration in determining whether a particular vector falls within the scope of the invention.

DNA constructs according to the invention may be easily be constructed from the component sequence elements using standard recombinant techniques well known in the art and described, for example, in F. M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994), as will be appreciated by one skilled in the art from the following detailed description of the invention and the accompanying Examples.

There follows a detailed description of DNA constructs according to the invention, with reference to the following schematic drawings in which:

Figures 1(a) to 1(e) are schematic representations of several different embodiments of the DNA construct according to the invention illustrating the relative positioning of the promoter and transcription terminator elements.

Figure 2(a) is a schematic representation of a prior

- 11 -

art vector included for comparison purposes.

Figures 2(b) to 2(e) are schematic representations of several further embodiments of the DNA construct  
5 according to the invention illustrating the use of different cloning sites in the inter-promoter region.

Referring to the Drawings, Figure 1(a) schematically illustrates a first DNA construct  
10 according to the invention which is a plasmid vector comprising two opposable promoters; a first promoter a) and second promoter b) flanking an inter-promoter region c), which inter-promoter region is downstream of the 3' of the first promoter, and downstream of the 3' end of the second promoter. The first promoter and the second promoter may be identical or different. This embodiment comprises a first transcription terminators e) and a second transcription terminator f) both of which are  
15 positioned within the inter-promoter region. In this embodiment, the first terminator and the second terminator are preferentially uni-directional terminators.

A DNA fragment may be inserted in the at least one cloning site d). Such fragment is subject to transcription directed by the first promoter a) and the second promoter b) (i.e. transcription of both strands), resulting in the generation of two RNA fragments which may combine to double-stranded RNA of  
20 the inserted DNA fragment (both *in vitro* and *in vivo*).

Any desired DNA sequence, such as a genomic DNA sequence, or a cDNA sequence or any other coding sequence, may be inserted in the at least one cloning site. Without being limited to any specific

- 12 -

explanation, it is assumed that when a) and e) form a functional combination, RNA polymerase which initiates transcription at a) will transcribe the inter-promoter region including the at least cloning site and the DNA  
5 fragment inserted in the at least cloning site and will be terminated when it reaches e). Similarly, RNA polymerase which initiates transcription at b) will transcribe the inter-promoter region including the at least one cloning site and the DNA fragment inserted  
10 in the at least one cloning site and will terminate when it reaches f). The terminators cause the RNA polymerase to pause, stop transcription and fall off the template. This prevents the unlimited transcription of the vector backbone, and reduces the  
15 unspecific transcription of non-essential DNA.

The inter-promoter region further comprises a sequence of nucleotides corresponding to a target for double-stranded RNA inhibition. This sequence is designated 'TF' for target fragment. It is this  
20 sequence which, when transcribed into dsRNA, will be responsible for specific double-stranded RNA inhibition of a target gene. The target fragment may be formed from a fragment of genomic DNA or cDNA from the target gene. Its precise length and nucleotide  
25 sequence are not material to the invention.

In the arrangement shown in Figure 1(a) the two terminators are positioned on either side of the TF within the inter-promoter region. Each of the terminators is positioned transcriptionally downstream  
30 of one of the promoters, the first terminator e) is transcriptionally downstream of first promoter a) and the second terminator f) is transcriptionally downstream of the second promoter b). Assuming that a) and e) form a functional combination, as described

above, RNA polymerase which initiates transcription at  
a) will transcribe the inter-promoter region up to and  
including TF and will be terminated when it reaches  
e). Similarly, RNA polymerase which initiates  
5 transcription at b) will transcribe the inter-promoter  
region up to and including TF on the opposite strand  
and will terminate when it reaches f). The  
terminators cause the RNA polymerase to pause, stop  
transcription and fall off the template. This  
10 prevents the unlimited transcription of the vector  
backbone, and reduces the unspecific transcription of  
non-essential DNA.

The transcripts generated from this vector may,  
depending on the precise placement of the terminators  
15 in the vector, be almost completely specific dsRNAs  
corresponding to the TF region. Through the direct  
placement of the terminator sequences at the  
downstream end of the TF region on both sides of the  
inserted DNA fragment, the amount of material  
20 transcribed is completely reduced to the  
template-specified sequences. Therefore, a higher  
amount of specific dsRNA is obtained. Furthermore the  
constructs are now also more stable, due to the  
non-transcription of the vector backbone. The latter  
25 characteristic (stability), combined with the now  
relatively higher specific transcription rate,  
provides a system adapted to synthesise higher amounts  
of specific short dsRNA strands. This proportionally  
higher amount of transcript, resulting in high  
30 concentrations of specific dsRNA, enhances the  
inhibitory effect in RNAi protocols. In RNAi  
protocols based on expression of dsRNA in a food  
organism, toxicity for the feeding organisms due to  
high RNA expression is brought to a minimal level by

use of this vector.

A specific example of a vector of the type illustrated in Figure 1(a), considered by the inventors to be the optimal arrangement for RNAi applications, is plasmid pGN9 described in the accompanying Examples. The transcriptional terminators used in pGN9 are T7 RNA polymerase specific terminators, since the vector contains two opposable T7 promoters. However, other systems could be used such as an expression system based on the T3 or SP6 promoter, terminator and polymerase or other prokaryotic or eukaryotic promoters and terminators.

Figure 1(b) illustrates schematically a further DNA construct according to the invention which is a plasmid vector comprising two opposable promoters a) and b) flanking an inter-promoter region c). This vector also comprises two transcription terminators e) and f) but in this arrangement the two terminators are positioned outside of the inter-promoter region, in fact the terminator elements now flank the two promoters. The arrangement is such that e) is transcriptionally downstream of a) whilst f) is transcriptionally downstream of b). Here again e) terminates the transcription initiated by a), whilst f) terminates the transcription initiated by promoter b). Placement of the terminators outside of d) allows the use of bi-directional terminators as well as uni-directional terminators, in contrast to the arrangement in Figure 1(a) where uni-directional terminators are preferred because of the placement of the terminators between the promoters. A number of bi-directional terminators which could be used in accordance with the invention are known in the art.

- 15 -

Generally these are observed to be polymerase non-specific.

The embodiment shown in Figure 1(b) provides essentially the same advantages as that shown in 5 Figure 1(a) over the prior art vectors for dsRNA production. The vector shown in Figure 1(b) will lead to the production of transcripts which are slightly longer, including the promoter regions. This relatively small difference in the length of the 10 transcript and hence the formed dsRNA will not severely affect the efficacy in an RNAi system.

The position of the terminators and promoter in the example as shown in figure 1 (b) are preferably placed at close proximity, such that the 5' end of the 15 promoters are separated from the 3' end of the transcription terminators by no more than 2000 nucleotides, preferably no more than 1000 nucleotides, more preferably no more than 500 nucleotides, even more preferably no more than 200 nucleotides, 20 especially preferably no more than 100 nucleotides, more especially preferably no more than 50 nucleotides, even more especially preferably no more than 20 nucleotides, particularly preferably no more than 10 nucleotides, more particularly preferably no 25 more than 6 nucleotides.

Figure 1(c) illustrates schematically a further DNA construct according to the invention which is a plasmid vector comprising two opposable promoters a) 30 and b) flanking an inter-promoter region c). In this embodiment one terminator (in this case e)) is positioned within the c) and the other (f)) is positioned outside c). Again, e) terminates transcription initiated by a) and f) terminates

- 16 -

transcription initiated by b). This arrangement may provide a useful solution to the problem of one of the terminators interfering with polymerase activity in the other direction (e.g. f) interferes with b) initiated transcription). This vector essentially provides the same advantages as the vector variations shown in Figure 1(a) and Figure 1(b) over the prior art. The relatively small difference in the length of the transcript due to the transcription of one of the promoters will not significantly affect the efficacy in RNAi systems. This more particularly the case when the terminator that is located outside of the inter-promoter region c) is at close proximity of the promoter, as defined above.

Figures 1(d) and 1(e) illustrate schematically two further DNA constructs according to the invention which are both plasmid vectors comprising two opposable promoters a) and b) flanking an inter-promoter region c). These embodiments comprise a single terminator only. In the arrangement shown in Figure 1(d) a single terminator e) which terminates transcription from a) is placed outside of c). The placement of the terminator outside of the IPR allows the use of both a bi-directional terminator or a uni-directional terminator in this system. In the embodiment shown in Figure 1(d) e) is placed within the c). a) should therefore preferably be a uni-directional terminator.

Further embodiments of the DNA construct according to the invention are illustrated schematically in Figures 2(b) to 2(e).

These embodiments are all plasmid cloning vectors, based upon the optimal arrangement of promoters and terminators shown in Figure 1(a), and

- 17 -

described above, containing cloning sites to facilitate the insertion of a DNA fragment into the at least one cloning site.

These embodiments are all plasmid cloning  
5 vectors, based upon the optimal arrangement of promoters and terminators shown in Figure 1(a), containing cloning sites to facilitate the insertion of a target DNA fragment into the inter-promoter region.

10 Figure 2(a), which is a schematic representation of a prior art cloning vector, is included for comparison purposes. This vector comprises two opposable promoters a) and b), which may be identical or different, flanking a multi-cloning site (MCS).

15 Figure 2(b) illustrates a first type of plasmid cloning vector according to the invention. The vector contains a first opposable promoter a) and a second opposable promoter b) flanking an inter-promoter region. The inter-promoter region can further be defined as: the DNA region between the 3' end of the first promoter and the 3' end of the second promoter, and which is downstream of the first promoter, and which is downstream of the second promoter, and which preferably does not contain the 5' end of the first 20 promoter and of the second promoter. The inter-promoter region further comprises terminators e) and f) flanking a multi-cloning site MCS. The MCS comprises at least one individual restriction sites, and preferably more than one 25 restriction site as known in the art, any of which may be used for insertion of a DNA fragment.

30 Figure 2(c) illustrates a further type of plasmid cloning vector according to the invention. This vector again contains opposable promoters a) and b)

- 18 -

flanking an inter-promoter region comprising terminators e) and f). In this embodiment, a) and b) flank a cloning site which is adapted for facilitated cloning of PCR fragments, comprising a stuffer DNA 5 flanked by two identical restriction sites, in this case BstXI sites. The specific sequence of the stuffer DNA is not essential, provided that said stuffer DNA does not interfere with the desired effect and/or the desired activity of the DNA constructs of the 10 invention. A specific example of a vector according to this aspect of the invention described herein is plasmid pGN29.

The cloning of PCR products using BstXI recognition sites and BstXI adaptors is generally 15 known in the art. The BstX1 adaptors are commercially obtained, such as from Invitrogen (Groningen, the Netherlands). These adaptors are non-palindromic adapters designed for easier and efficient cloning of PCR products into vectors. These use of these adaptors 20 reduces the concatemerization of adapters or insert DNA by having non-complementary (CACA) overhangs. The stuffer DNA is included merely to allow easy differentiation between BstXI cut and uncut vector on the basis of size. Its precise length and sequence 25 are not of importance.

Figure 2(d) illustrates a further type of plasmid cloning vector according to the invention. This vector again contains opposable promoters a) and b) flanking an inter-promoter region comprising 30 terminators e) and f). In this embodiment, a) and b) flank a cloning site which facilitates "High Throughput" cloning based on homologous recombination rather than restriction enzyme digestion and ligation.

As shown schematically in Figure 2(d), the cloning

- 19 -

site comprises attR1 and attR2 recombination sites from bacteriophage lambda flanking a gene which is lethal to *E. coli*, in this case the ccdB gene.

An alternative cloning method of DNA fragments 5 into this vector, (not shown in Figure 2 (d)), consists of a variant of this vector, wherein the ccdB DNA sequence further comprises at least one unique restriction site, preferably the at least unique restriction site is a blunt-end restriction site, such 10 as a SrfI restriction site. Insertion of a DNA fragment in the at least unique restriction, results in inactivation of the ccdB gene, and hence in inactivation of the lethal ccdB gene.

A further variant of a vector a shown in Figure 15 2(d) in which the attR1 and the attE2 are not present. Such a vector comprises an at least one cloning site, said at least one cloning site consisting of a ccdB sequence, said ccdB sequence further comprising at least one unique restriction site, preferably the at 20 least unique restriction site is a blunt-end restriction site, such as a SrfI restriction site. Insertion of a DNA fragment in the at least unique restriction, results in inactivation of the ccdB gene, and hence in inactivation of the lethal ccdB gene.

25 These cloning sites comprising the ccdB nucleotide sequence and/or the attR sites (R1 and/or R2) are derived from the Gateway™ cloning system commercially available from Life Technologies, Inc. The Gateway™ cloning system has been extensively 30 described by Hartley et al. in WO 96/40724 (PCT/US96/10082). A specific example of a vector according to this aspect of the invention described herein is pGN39.

- 20 -

Figure 2(e) and 2(f) illustrate a still further type of plasmid cloning vector according to the invention. This vector again contains opposable promoters a) and b) flanking an inter-promoter region 5 c) comprising terminators e) and f). In the embodiment shown in Figure 2(e), e) and f) flank a cloning site which facilitates "high throughput" cloning of PCR products by TA<sup>TM</sup> cloning. This cloning site comprises a stuffer DNA flanked by two identical 10 restriction sites for an enzyme which generates overhanging T nucleotides. In this case the restriction sites are XcmI sites, but other sites which are cleaved to generate overhanging T nucleotides could be used with equivalent effect. The 15 overhanging T nucleotides facilitate cloning of PCR products which have a overhanging A nucleotide. This principle is known as TA<sup>TM</sup> cloning. The cut vector with overhanging T nucleotides can be "topomerized" to generate a cloning vector of the type shown 20 schematically in Figure 2(f), by linking the enzyme topoisomerase to the overhanging T nucleotides. The resulting vector also facilitates the cloning of PCR products by the principle known as TOPO<sup>TM</sup> cloning.

Both the TOPO<sup>TM</sup> and TA<sup>TM</sup> cloning systems, although 25 not for the vectors described in this invention, are commercially available from Invitrogen. The TOPO<sup>TM</sup> cloning system has extensively been described by Shuman in WO 96/19497 (PCT/US95/16099). The TA<sup>TM</sup> cloning system has extensively been described by 30 Hernstadt et al. in WO 92/06189 (PCT/US91/07147).

It will be readily appreciated by the skilled reader that whilst Figures 2(b)-2(f) illustrate the inclusion of different cloning sites into a vector of the type illustrated in Figure 1(a), these cloning

- 21 -

sites could be included into any of the DNA constructs of the invention, including those illustrated schematically in Figures 1(b) to 1(e)

5   Application of the DNA constructs of the invention in RNAi technology.

As aforementioned, a major application of the DNA constructs/vectors of the invention is in the production of double stranded RNA for use in RNAi 10 technology. In particular, the constructs are useful in *in vivo* RNAi protocols in the nematode worm *C. elegans*.

In *C. elegans*, RNAi has traditionally been performed by injection dsRNA into the worm. Fire et 15 al. describes these methods extensively in International Application No. WO 99/32619. In short, both strands of RNA are produced *in vitro* using commercially available *in vitro* transcription kits. Both strands of RNA are allowed to form dsRNA, after 20 which the dsRNA is injected into *C. elegans*.

The new vector system developed by the present inventors is a drastic improvement on this traditional method. First, the RNA can be produced in one step, for instance by using two identical promoters such as 25 in the vector pGN9. Second, and more important, due to the presence of terminators the transcripts, and hence the formed dsRNA, will be more specific as only the cloned target fragment will be transcribed. This will result in a more efficient RNAi.

30   A further method to perform RNAi experiments in *C. elegans* has been described by Plaetinck et al. in WO 00/01846. In this method *C. elegans* worms are fed with bacteria which produce dsRNA. The dsRNA passes the gut barrier of the worm and induces the same RNAi

- 22 -

as if the dsRNA has been injected. For these experiments, the preferred *E. coli* strain is HT115 (DE3), and the preferred *C. elegans* strain is nuc-1;gun-1. The improved vectors provided by the 5 invention also improve the efficiency of RNAi in this method, as shown in the example below, as only effective dsRNA is produced.

Another method to perform RNAi has also been described by Plaetinck et al. in WO 00/01846. In 10 short, this method is based on the production of dsRNA in the worm itself. This can be done by using worm promoters in the described vectors, or by using a transgenic worm expressing a polymerase specific for non-*C. elegans* promoters present in the vector, such 15 that this polymerase drives transcription of the dsRNA. The promoters will preferentially be selected from the known bacteriophage RNA promoters, such as T7 or T3 or SP6 RNA promoters, which provide the advantage of a high level of transcription dependent 20 only on the binding of the cognate polymerase.

Plasmid vector DNA can be introduced into the worm by several methods. First, the DNA can be introduced by the traditional injection method (Methods in Cell Biology, Vol 48, *C. elegans* Modern 25 Biological Analysis of an organism, ed. by Epstein and Shakes). Second, the DNA can be introduced by DNA feeding. As has been shown by Plaetinck et al. in WO 00/01846, plasmid DNA can be introduced into the worm by feeding the worm with an *E. coli* strain that 30 harbors the plasmid. Preferentially the *E. coli* strain is OP50, or MC1061 or HT115 (DE3) but any other strain would suit for this purpose. The *C. elegans* strain is preferentially a nuc-1 mutant strain or a nuc-1; gun-1 strain. The plasmid DNA from the *E. coli*

passes the gut barrier and is introduced into the nematode, resulting in the expression of dsRNA. As with the other RNAi methods described above, the use of the new vector system will enhance the RNAi by the 5 production of only specific dsRNA.

The invention will be further understood with reference to the following experimental Examples, together with the following additional Figures in 10 which:

Figure 3 is a representation (plasmid map) of pGN1.

15 Figure 4 is a representation (plasmid map) of pGN9.

Figure 5 illustrates the nucleotide sequence of a fragment of plasmid pGN1, annotated to show the positions of the opposable T7 promoters.

20 Figure 6 depicts the nucleotide sequence of the T7 transcription terminator.

25 Figure 7 illustrates the sequences of oligonucleotides oGN27, oGN28, oGN29 and oGN30 used to insert T7 transcription terminators into pGN1. The positions of the T7 pol terminator sequences and of various restriction sites are marked.

30 Figure 8 illustrates the nucleotide sequence of a fragment of plasmid pGN9, annotated to show the positions of the opposable T7 promoters and the T7 transcription terminators.

- 24 -

Figure 9 (a) is a representation (plasmid map) of pGN29; (b) is a representation (plasmid map) of pGN39; (c) is a representation (plasmid map) of the plasmid TopoRNAi.

.5

Figure 10 shows the complete nucleotide sequence of plasmid pGN9.

10 Figure 11 shows the complete nucleotide sequence of plasmid pGN29.

Figure 12 shows the complete nucleotide sequence of plasmid pGN39.

15 Figure 13 shows the complete nucleotide sequence of plasmid TopoRNAi.

Figure 14 shows the complete sequence of plasmid pGN49A.

20

Figure 15 shows the complete sequence of plasmid pGN59A.

25

Figure 16 is a representation (plasmid map) of pGN49A.

Figure 17 is a representation (plasmid map) of pGN59A.

30

**Example 1-Vector construction.**

5 The starting point for construction of the vectors exemplified herein was plasmid pGN1. This plasmid, described in the applicant's co-pending International Application No. WO 00/01846, contains two opposable T7 promoters flanking a multi-cloning site.

10 Vector construction was carried out according to standard molecular biology techniques known in the art and described, for example, in F. M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994).

15

**1) Construction of pGN9**

pGN1 was first digested with restriction enzymes EcoRI and KpnI. Oligonucleotides oGN27 and oGN28 (Figure 7) were annealed to generate a double stranded fragment which was then ligated into the EcoRI/KpnI cut vector. The resulting plasmid was re-digested with XbaI and HindIII. Oligonucleotides oGN29 and oGN30 were annealed to generate a double-stranded fragment which was then annealed into the XbaI/HindIII cut vector. The resulting vector was designated pGN9 (Figures 4 and 10).

**2) Construction of further cloning vectors**

pGN29 (Figure 9(a); Figure 11) was generated by 30 replacing the MCS in pGN9 with a stuffer DNA flanked by BstXI sites. BstXI adapters are commercially available from Invitrogen (Groningen, the Netherlands).

- 26 -

pGN39 (Figure 9 (b); Figure 12) generated by following steps; pGN29 was digested with BstXI. BstXI adapters (Invitrogen, Groningen, The Netherlands) were ligated  
5 to Cassette A provided by the GATEWAYTM system (Life Technologies, Inc.). Cassette A contains attR1, CmR,  
CcdA, CcdB, attR2. The Cassette A with the adapters where then ligated into the digested pGN29, resulting  
in pGN39A. pGN39A contains a unique SrfI site in the  
10 ccdB gene.

The TopoRNAi vector (figure 9 (c); figure 13) was generated in the following way; pGN29 was digested with BstXI. Using PCR with the primers oGN103 and  
15 oGN104 and template pCDM8 (Invitrogen, Groningen, The Netherlands), a stuffer was generated which includes XcmI sites. Onto the PCR product, BstXI adapters were ligated, and the resulting ligation product was ligated in the BstXI digested pGN29 vector resulting  
20 in the TopoRNAi vector.

oGN103: 5' TACCAAGGCTAGCATGGTTATCACTGATAAGTTGG 3'

oGN104: 5' TACCAAGGCTAGCATGGCCTGCCTGAAGGCTGC 3'

25 PGN49A was constructed to insert an additional unique non-blunt restriction site and to delete the CmR gene pGN39. Overlap PCR was used. A first PCR was performed with primers oGN126 and oGN127 and PGN39A as template. Using primers oGN128 and oGN129 and the same template a  
30 second fragment was generated. Overlap PCR using the resulting fragments and primers oGN126P and oGN129P resulted in a final PCR product. To this final PCR

- 27 -

product, BstXI adapters were ligated, and the ligation product was digested with BstXI. The resulting vector was designated pGN49A.

5 A control vector was generated to test the efficiency of the pGN49A cloning vector, such vector should contain the T7 promoters, but not the T7 terminators. For this, the XbaI insert of pGN49A was isolated and cloned in pGN1 digested with the same restriction 10 enzyme. The resulting vector was designated pGN59A.

oGN126 pGATCTGGATCCGGCTTACTAAAAGCCAGATAACAGTATGC  
oGN127 GGAGACTTTATCGCTTAAGAGACGTGCACTGCCAGGGGGATCACC  
oGN128:  
15 CCAGTGCACGTCTCTTAAGCGATAAAAGTCTCCGTGAACTTACCCGGTGG  
oGN129 pGCTGTGTATAAGGGAGCCTGACATTATATTCCCCAG

Example 2-To illustrate the usefulness of the improved vectors in RNA.

20 This experiment was designed to illustrate the improved efficiency of the improved vectors of this invention in double-stranded RNA inhibition, as compared to the vectors known from the prior art. A significant increase on the efficacy of the system 25 could be expected, as more effective dsRNA was produced and hence RNAi performed better. The experimental system for this proof of concept experiment was to measure *C. elegans* rescue at 25°C in nuc-1 / pha-1(e2123)ts *C. elegans* mutants by RNAi of 30 sup35 using dsRNA feeding of pGN-2 (-terminator) and pGN-12 (+ terminator), with PGN-1 (empty vector) as a control and dilutor. The pha-1 ts / sup-35 mutation has extensively been described by Schnabel in WO

99/49066.

The *nuc-1* mutation in *C. elegans* provides for a *C. elegans* strain exhibiting better uptake abilities,  
5 such as the uptake of the dsRNA complexes than wild type *C. elegans*. This mutant is deleted in the major DNAse enzymes, and inventors have proven in earlier co-pending applications that this *C. elegans* strain results in enhanced RNAi by feeding the nematode with  
10 dsRNAs.

The *pha-1(e2123)ts* mutation provides a mutant *C. elegans* strain with a phenotype of survival at 15°C and lethality at 25°C. This lethality is suppressible  
15 by the inhibition of *sup-35* expression. RNAi of *sup-35* should thus facilitate the rescue of *pha-1(e2123)ts* at 25°C. The vectors of the present invention, when expressing dsRNA from *sup-35*, should increase the efficacy of *sup-35* RNAi in rescuing *pha-1(e2123)ts*  
20 mutants at 25°C, compared to vectors that do not contain the terminators.

Vector pGN1 was used as empty vector. Vector pGN2 (-terminator) is a vector harboring *sup-35* DNA and  
25 expressing *sup-35* dsRNA when introduced in the appropriate host, the vector has no transcriptional terminators inserted. Vector pGN12 (+ terminator) is a vector as described above, containing the transcriptional terminators, and hence resulting in  
30 improved dsRNA production when introduce into an appropriate host. Thus, this vector has two unidirectional transcriptional terminators, both placed inside of the inter-promoter region, and flanking the *sup-35* fragment. Use of the latter

- 29 -

vector was expected to increase the efficacy of the system, here meaning a better rescue (survival) of pha-1(e2123)ts mutants at 25°C.

5   **Experimental conditions**

12-well micro-titer plates were filled with approximately 2 ml of NGM agar per well.

(1 liter of NGM agar: 15g Agar, 1g peptone, 3g NaCl, 1ml cholesterol solution (5 mg/ml in EtOH), with 10 sterile addition after autoclaving of 9.5 ml 0.1M CaCl<sub>2</sub>, 9.5 ml 0.1 ml MgSO<sub>4</sub>, 25 ml 1M KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> buffer pH 6, Ampicillin (100 µg/l), 5ml 0,1M IPTG and 5 ml nystatin solution (dissolved 10 mg/ml in 1:1 EtOH:CH<sub>3</sub>COONH<sub>4</sub> 7.5 M)

15

The dried plates were spotted with approximately 50 µl of an overnight culture of bacteria HT115 (DE3) (Fire A, Carnegie Institution, Baltimore, MD) transformed with the plasmids. Individual nematodes at the L4 growth stage were then placed in single wells at day 1. In each well 1 nematode (P1). At day two, the P1 nematodes were placed to a new well and left to incubate for a day. The same procedure was repeated at day 3. All plates were further incubated at 25°C to 20 allow offspring to be formed. Sup35 RNAi induced 25 survival (rescue) was measured by counting the offspring.

**Results**

30 RNAi experiment in *C. elegans* nuc-1/pha-1(e2123)ts mutants by feeding with *E. coli* expressing sup-35 dsRNA.

- 30 -

**Set up:**

pGN1 as control

pGN2 (sup 35 - Term.)

pGN12 (sup 35 + Term.)

5

pGN2 + pGN1 dilutions 1/2, 1/4, 1/8, 1/16, 1/32

pGN12 + pGN1 dilutions 1/2, 1/4, 1/8, 1/16, 1/32

10 Conditions:

Incubation temperature 25°C

**Readout:**

Count offspring (adult hermaphrodites)

**pGN1 (control)**

Day 1	0	0	0	0
Day 2	0	0	0	0
Day 3	0	0	0	0

**pGN2 (undiluted)**

Day 1	12	4	48	32
Day 2	24	23	80	85
Day 3	5	0	9	16

**pGN12 (undiluted)**

Day 1	16	29	37	14
Day 2	27	22	57	2
Day 3	1	2	4	1

**pGN 2+1, 1/2 dilution**

Day 1	0	7	0	2
Day 2	9	10	0	3
Day 3	0	2	0	0

**pGN 12+1, 1/2 dilution**

Day 1	22	28	103	61
Day 2	36	45	53	40
Day 3	3	3	25	1

- 31 -

pGN 2+1, 1/4 dilution

Day 1	28	23	0	0
Day 2	6	3	0	0
Day 3	0	0	0	0

pGN 12+1, 1/4 dilution

Day 1	*	6	36	5
Day 2		24	55	3
Day 3				

pGN 2+1, 1/8 dilution

Day 1	0	0	4	0
Day 2	0	0	11	0
Day 3	0	0	0	0

pGN 12+1, 1/8 dilution

Day 1	31	12	16	38
Day 2	4	5	37	4
Day 3	0	0	2	1

pGN 2+1, 1/16 dilution

Day 1	0	0	0	0
Day 2	0	0	0	1 little
Day 3	0	0	0	0

pGN 12+1, 1/16 dilution

Day 1	1	0	0	0
Day 2	2	0	0	1
Day 3	0	1	1	1

pGN 2+1, 1/32 dilution

Day 1	0	0	0	0
Day 2	0	0	0	0
Day 3	0	0	0	0

pGN 12+1, 1/32 dilution

Day 1	0	0	1	0
Day 2	0	L2	3	0
Day 3	2	0	L3- L4	0

**Conclusions**

As expected, worms fed by bacteria harboring pGN1, did not result in the viable offspring, due to the lethal effect of the pha-1 mutation at this temperature.

5 Feeding the nematodes with *E. coli* harboring pGN2 or pGN12 both result in viable offspring. This is due to the feeding of the worm with dsRNA from sup-35. The remarkable difference between the two feeding experiments can be seen in the dilution series. When  
10 diluting the bacteria harboring pGN2 with bacteria harboring pGN1, the number of offspring diminishes drastically, even at a low dilution of one to two. This dilution series indicates that high levels of dsRNA are needed to have a proper RNAi induction. In  
15 the feeding experiment with bacteria harboring pGN12, significant offspring is still observed at a dilution of one to eight. This indicates that in the bacteria harboring pGN12, much more effective dsRNA is formed. This experiment clearly shows that the addition of  
20 terminator sequences in vectors to express dsRNA as described above provide a significant advantage in the generation of RNAi.

Example 3: Comparison of RNAi efficiency of vectors  
25 with and without T7 terminators (pGN49 vs pGN59)

Three different genes have been cloned in the vectors pGN49A and pGN59A. The cloning was performed by amplifying the gene fragments with PfuI DNA polymerase  
30 producing blunt ends, facilitating cloning in these vectors. These PCR fragments were cloned into the vectors digested with SrfI. Correct fragment insertion of the clones was checked by PCR. The fragments are chosen such that ds expression and RNAi results in a

- 33 -

lethal phenotype of the offspring. This procedure allows to compare fast and easy the efficiency of the two vectors pGN49 and pGN59 in RNAi.

plasmid	Gene (acedb)	Vector backbones
pGW5	B0511.8	pGN49A
pGW9	C01G8.7	pGN49A
pGW11	C47B2.3	pGN49A
pGW17	B0511.8	pGN59A
pGW21	C01G8.7	pGN59A
pGW23	C47B2.3	pGN59A

All the plasmids (pGW-series) are transformed in *E.coli* AB301-105 (DE3) bacteria by standard methodology. The bacteria are then grown in LB/amp at 37°C for 14-18h.

25 These cultures were centrifuged and the bacterial pellet dissolved in S-complete buffer containing 1mM IPTG and 100 µg/µl ampicilin.

30 In 96 well plates containing 100 µl S-complete (containing 1 mM IPTG and 100 µg/µl ampicilin final concentration) and 10 µl of bacteria solution, 3 nematodes were added at each well, the nematodes were at the L1 growth stage.

The plates were incubated at 25°C for 5-6 days. Each

- 34 -

day the plates are inspected for development of the larvae and the production of F1 offspring.

### 5 Results

The RNAi was performed in eight-fold for each constructed plasmid. The results show that when T7 terminators are inserted into the vector backbone, the expected phenotype gives a 100% occurrence. When T7 terminators are not used the reproducibility can decrease up to 50%. As in the previous experiment, the results show that the addition of terminators significantly enhances RNAi performance.

#### DNA

fragment	B0511.8	B0511.8	C01G8.7	C01G8.7	C47B2.3	C47B2.3
Vector	pGN49A	pGN59A	PGN49A	pGN59A	pGN49A	pGN59A
Resulting						
plasmid	PGW5	PGW17	PGW9	PGW21	PGW11	PGW23
Percentage						
lethal	100	75	100	87.5	100	50
Percentage						
offspring	0	25	0	12.5	0	50

- 35 -

PCR fragment generated by the primers oGN103 and  
oGN104 on template pCDM8

TACCAAGGCT AGCATGGTTT ATCACTGATA AGTTGG  
5 ATAAGTTGGT GGACATATTA TGTTTATCAG TGATAAAAGTG TCAAGCATGA  
CAAAGTTGCA GCCGAATACA GTGATCCGTG CCGGCCCTGG ACTGTTGAAC  
GAGGTCGGCG TAGACGGTCT GACGACACGC AAACCTGGCGG AACGGTTGGG  
GGTGCAGCAG CGGGCGCTTT ACTGGCACTT CAGGAACAAAG CGGGCGCTGC  
TCGACGCACT GGCGAAGCC ATGCTGGCGG AGAACATACAC GCTTCGGTGC  
10 CGAGAGCCGA CGACGACTGG CGCTCATTTC TGATCGGGAA TCCCGCAGCT  
TCAGGCAGGC CCATGCTAGC CTGGTACCA GCACAATGG

Overlap PCR Fragment, which was used to generate  
15 pGN49A

gatctggatccggcttactaaaagccagataacagtatgcgtattgcgcgctg  
attttgcgtataagaatatatactgatatgtataccgaagtatgtcaaaaa  
gaggtgtgctatgaagcagcgtattacagtacagttgacagcgacagctatca  
20 gttgctcaaggcatatatgatgtcaatatctccgtctggtaagcacaaccatg  
cagaatgaagcccgtcgtctgcgtgccgaacgctggaaagcggaaaatcaggaa  
gggatggctgaggtcgcccggttattgaaatgaacggctctttgctgacgag  
aacaggactggtaatgcagttaaaggttacacctataaaagagagagccg  
ttatcgctgtttgtggatgtacagagtatattgacacgcggcgca  
25 cggatggtgatccccctggccagtgcacgtctttaagcgataaagtctccc  
gtgaactttaccgggtggtcataatcgggatgaaagctggcgcatgtac  
caccgatatggccagtgtgccgtctccgttatcgaaaagaagtggctgat  
ctcagccaccgcgaaaatgacatcaaaaacgccattaacctgatgttctgg  
gaatataaatgtcaggctcccttatacacagc

30

Claims:

1. A DNA construct comprising:
  - a) a first promoter and
  - 5 b) a second promoter,  
in which the first and second promoter are in opposite orientation to each other and define:
  - c) an inter-promoter region positioned downstream of the 3' end of the first promoter and downstream of  
10 the 3' end of the second promoter;  
and which DNA construct further comprises:
  - d) at least one cloning site positioned in the inter-promoter region; and
  - e) a first transcription terminator, positioned (as  
15 seen from the 3' end of the first promoter)  
downstream of the first promoter and downstream of the at least one cloning site, wherein the first transcription terminator is operably linked to the first promoter.
- 20 2. A DNA construct according to claim 1, further comprising:
  - f) a second transcription terminator positioned (as seen from the 3' end of the second promoter)  
25 downstream of the second promoter and downstream of the at least one cloning site.  
wherein the second transcription terminator is operably linked to the second promoter.
- 30 3. A DNA construct according to claim 1 or 2, in which the first transcription terminator is positioned in the inter-promoter region.

4. A DNA construct according to claim 1 or 2, in which the first transcription terminator is positioned (as seen from the 3' end of the first promoter) downstream of the first promoter, downstream of the at least one cloning site, and downstream of the 5' end of the second promoter.
5. A DNA construct according to any one of claims 2, 10 3 or 4, in which the second transcription terminator is positioned in the inter-promoter region.
6. A DNA construct according to any of claims 2, 3 15 or 4 in which the second transcription terminator is positioned (as seen from the 3' end of the second promoter) downstream of the second promoter, downstream of the at least one cloning site, and downstream of the 5' end of the first promoter.
7. A DNA construct according to any one of claims 4, 25 5 or 6, in which the 3' end of the first transcription terminator is separated from the 5' end of the second promoter by no more than 2000 nucleotides, preferably no more than 1000 nucleotides, more preferably no more than 500 nucleotides, even more preferably no more than 200 nucleotides, especially preferably no more than 100 nucleotides, more especially preferably no more than 50 nucleotides, even more especially preferably no more than 20 nucleotides, particularly preferably no more than 10

nucleotides, more particularly preferably no more than 6 nucleotides.

8. A DNA construct according to any one of claims 6 or 7, in which the 3' end of the second transcription terminator is separated from the 5' end of the first promoter by no more than 2000 nucleotides, preferably no more than 1000 nucleotides, more preferably no more than 500 nucleotides, even more preferably no more than 200 nucleotides, especially preferably no more than 100 nucleotides, more especially preferably no more than 50 nucleotides, even more especially preferably no more than 20 nucleotides, particularly preferably no more than 10 nucleotides, more particularly preferably no more than 6 nucleotides.
9. A construct according to any one of the preceding claims wherein the first and the second promoter are identical.
10. A DNA construct according to any one of the claims 1 to 7 wherein the first and the second promoter are non-identical.
11. A DNA construct according to claims 8 or 9 wherein the first promoter and the second promoter are independently chosen from T7, T3 or SP6 promoters.
12. A construct according to any one of the preceding claims wherein the cloning site comprises at

- 39 -

least one restriction site.

13. A DNA according to claim 11 wherein the cloning site comprises at least two restriction sites flanking a sequence of stuffer DNA.
14. A DNA construct according to claim 12 wherein the at least two restriction sites are identical.
- 10 15. A DNA construct according to any one of the claim 12 to 13 wherein the at least one restriction site or the at least two restriction sites restriction sites are *BstXI* sites.
- 15 16. A DNA construct according to any one of the claims 12 to 13 wherein the restriction sites are *XcmI* sites.
- 20 17. A DNA construct according to any one of the preceding claims wherein the cloning site further comprises *attR1* and *attR2* recombination sequences.
- 25 18. A DNA construct according to any one of the preceding claims wherein the cloning site further comprises a *ccdB* nucleotide sequence.
- 30 19. A DNA construct according to claim 17 wherein the *ccdB* nucleotide sequence further comprises at least one unique restriction site.
20. A DNA construct according to claim 18 wherein the at least one unique restriction site are blunt-end restriction sites.

21. A DNA construct according to claim 19 wherein the blunt-end restriction sites are *SrfI* sites.
- 5 22. A DNA according to any one of the preceding claims which further comprises:
  - g) a DNA fragment inserted in the at least one cloning site.
- 10 23. A DNA construct according to any one of the preceding claims which is a plasmid or vector.
24. A plasmid or vector as claimed in claim 23 having the nucleotide sequence illustrated in Figure 10,  
15 Figure 11, Figure 12, Figure 13, Figure 14, or Figure 15.
25. Use of the DNA construct according to any one of the preceding claims for the production of  
20 double-stranded RNA for RNA inhibition.
26. A bacterial strain harbouring the DNA construct according to any one of the preceding claims.
- 25 27. A bacterial strain according to claim 26, wherein said bacteria strain is an *E. coli* strain.
28. Use of the bacterial strain according to claims  
26 or 27 for the production of double-stranded  
30 RNA for RNA inhibition.

FIG. 1(a)

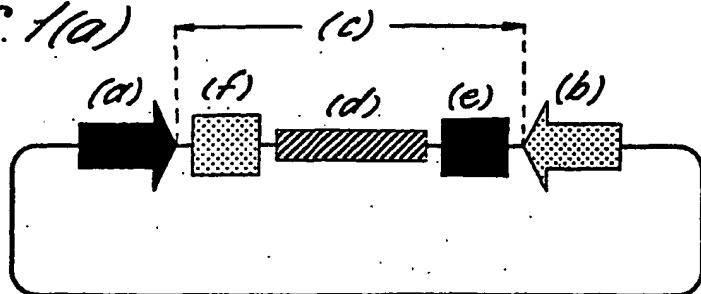


FIG. 1(b)

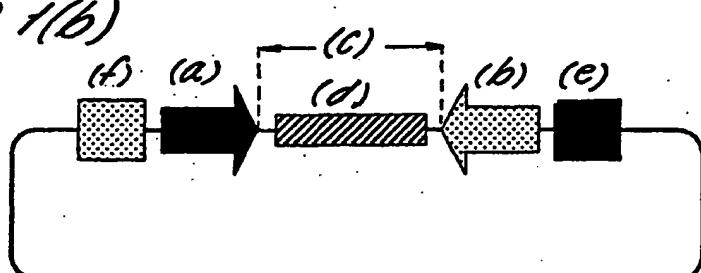
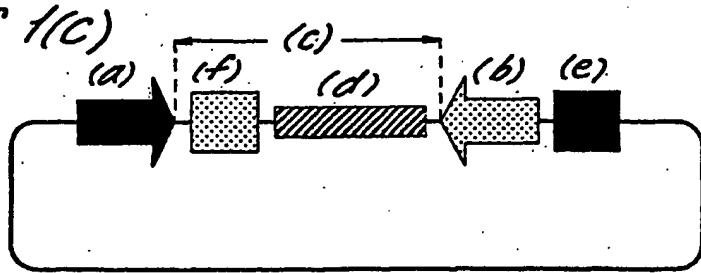


FIG. 1(c)



(a) : promoter 1  
(b) : promoter 2

(c) : Terminator 1  
(d) : Terminator 2

FIG. 1(d)

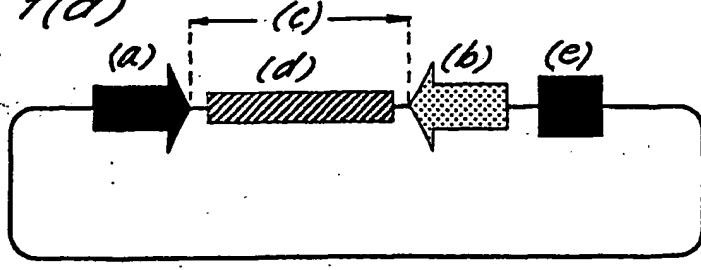


FIG. 1(e)

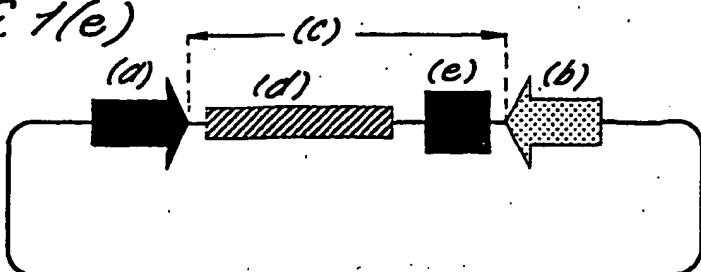


FIG. 2(a)

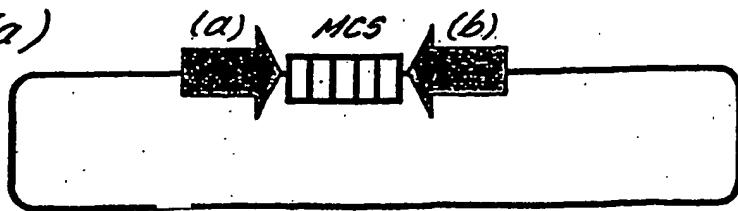


FIG. 2(b)

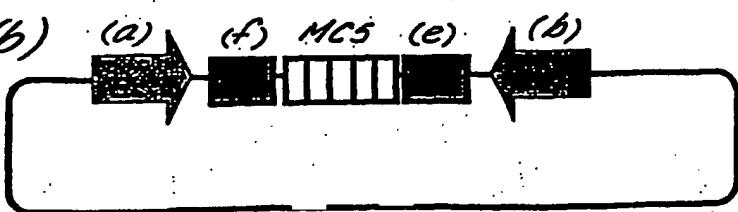


FIG. 2(c)

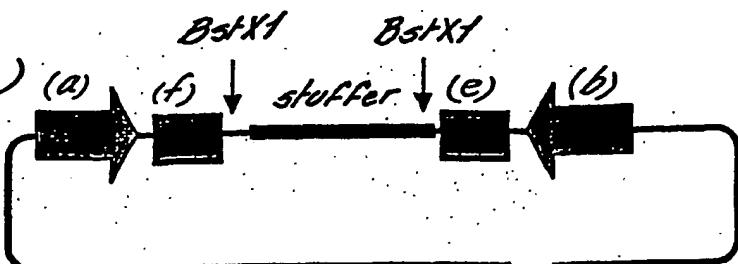


FIG. 2(d)

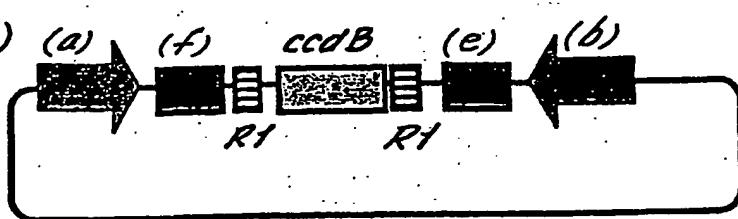


FIG. 2(e)

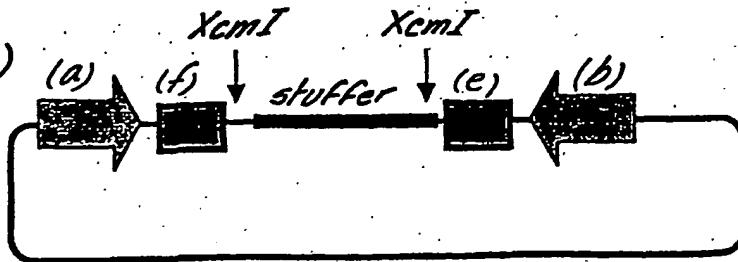
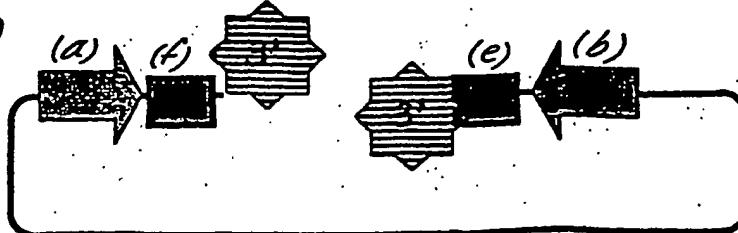


FIG. 2(f)



## Construction RNAi vector with T7 terminators

FIG. 3.

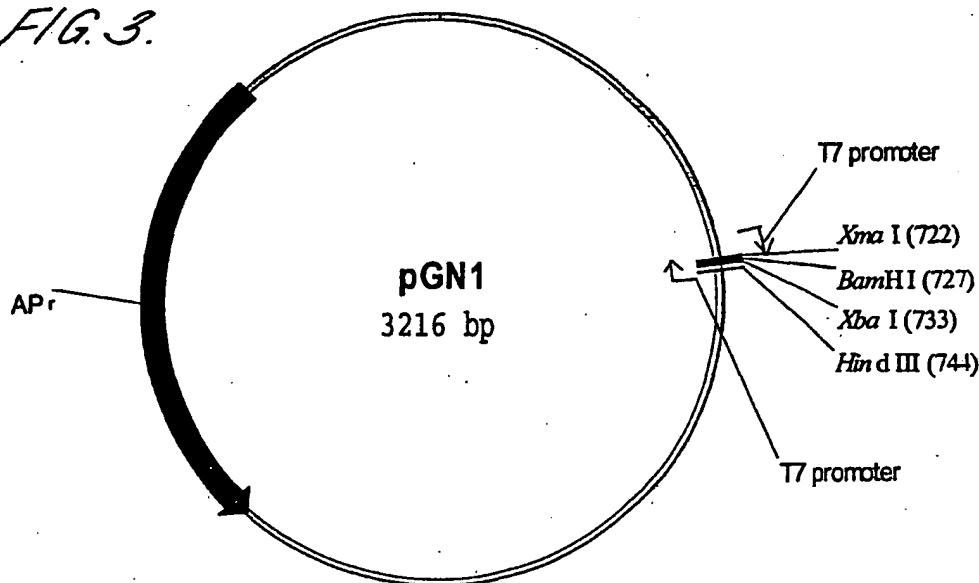


FIG. 4.

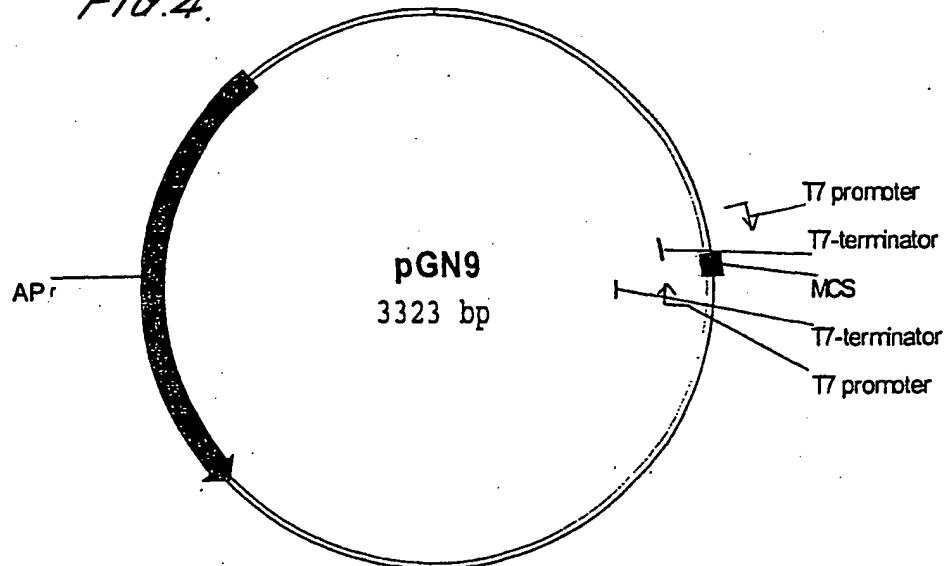


FIG. 5.

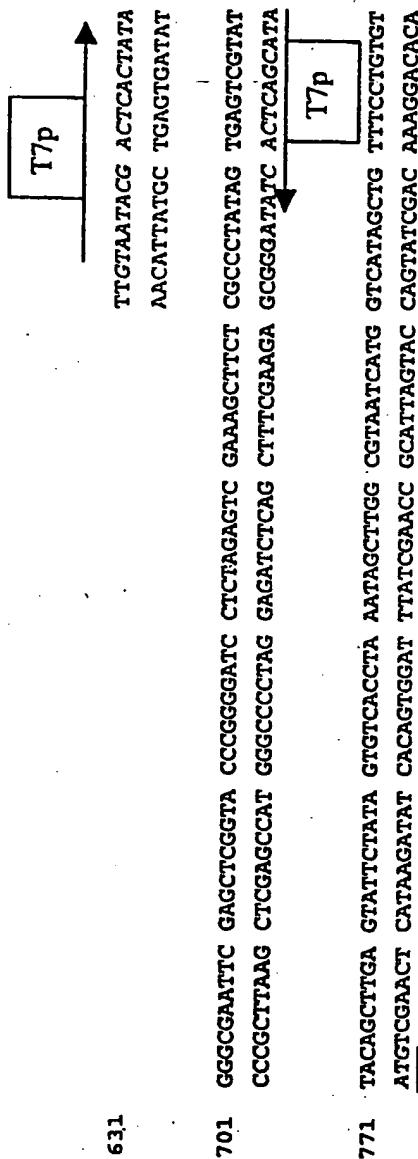


FIG. 6.

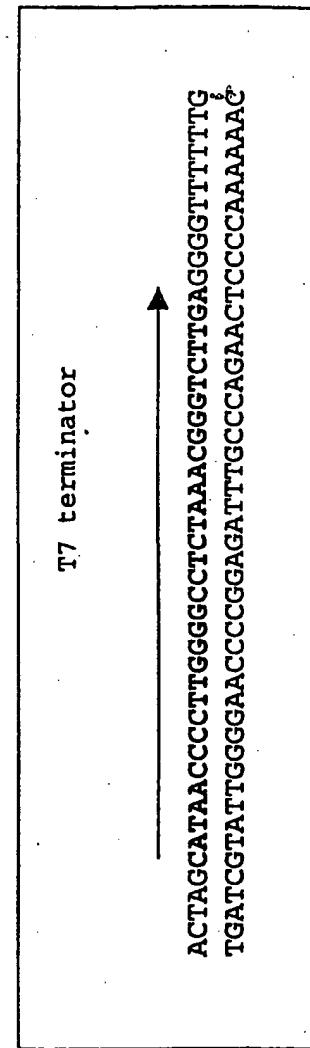
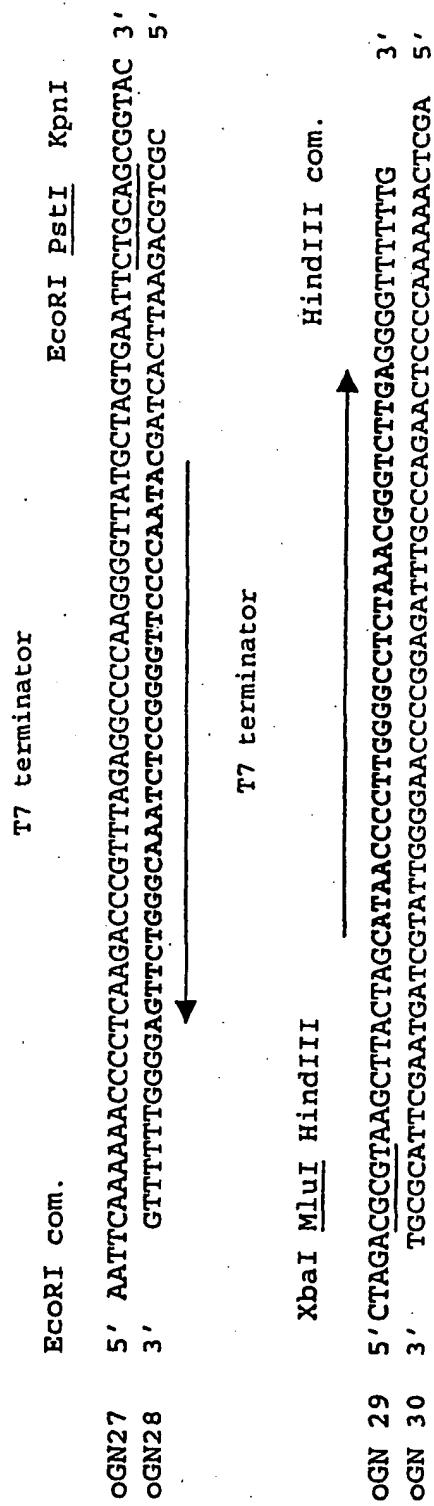


FIG. 7



*FIG. 8.*

631

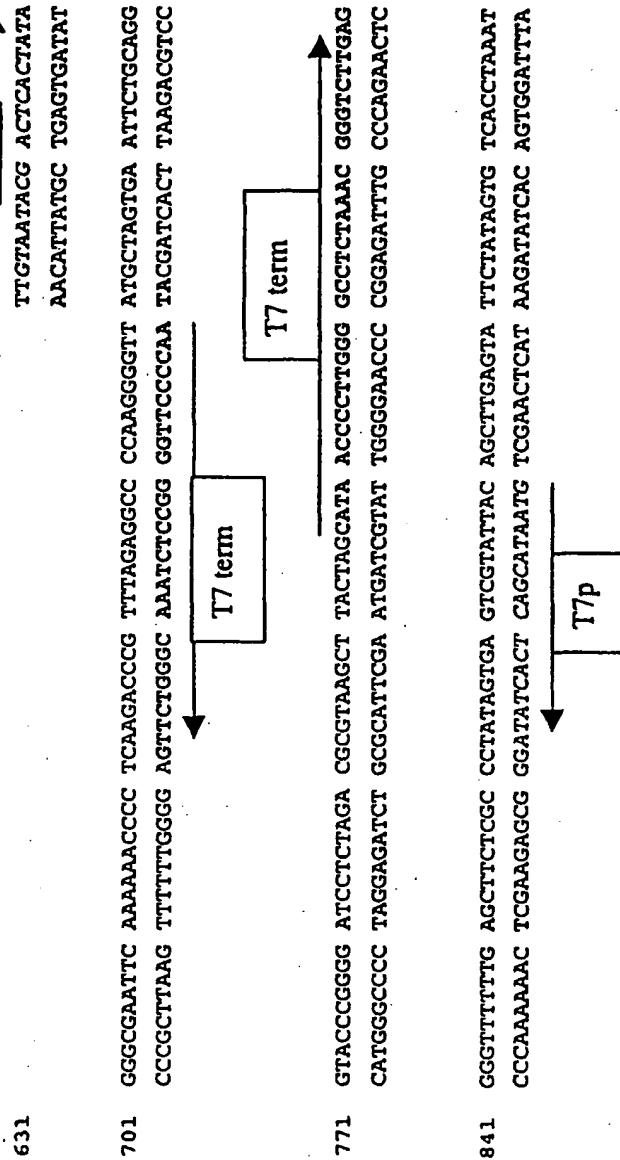


FIG. 9.

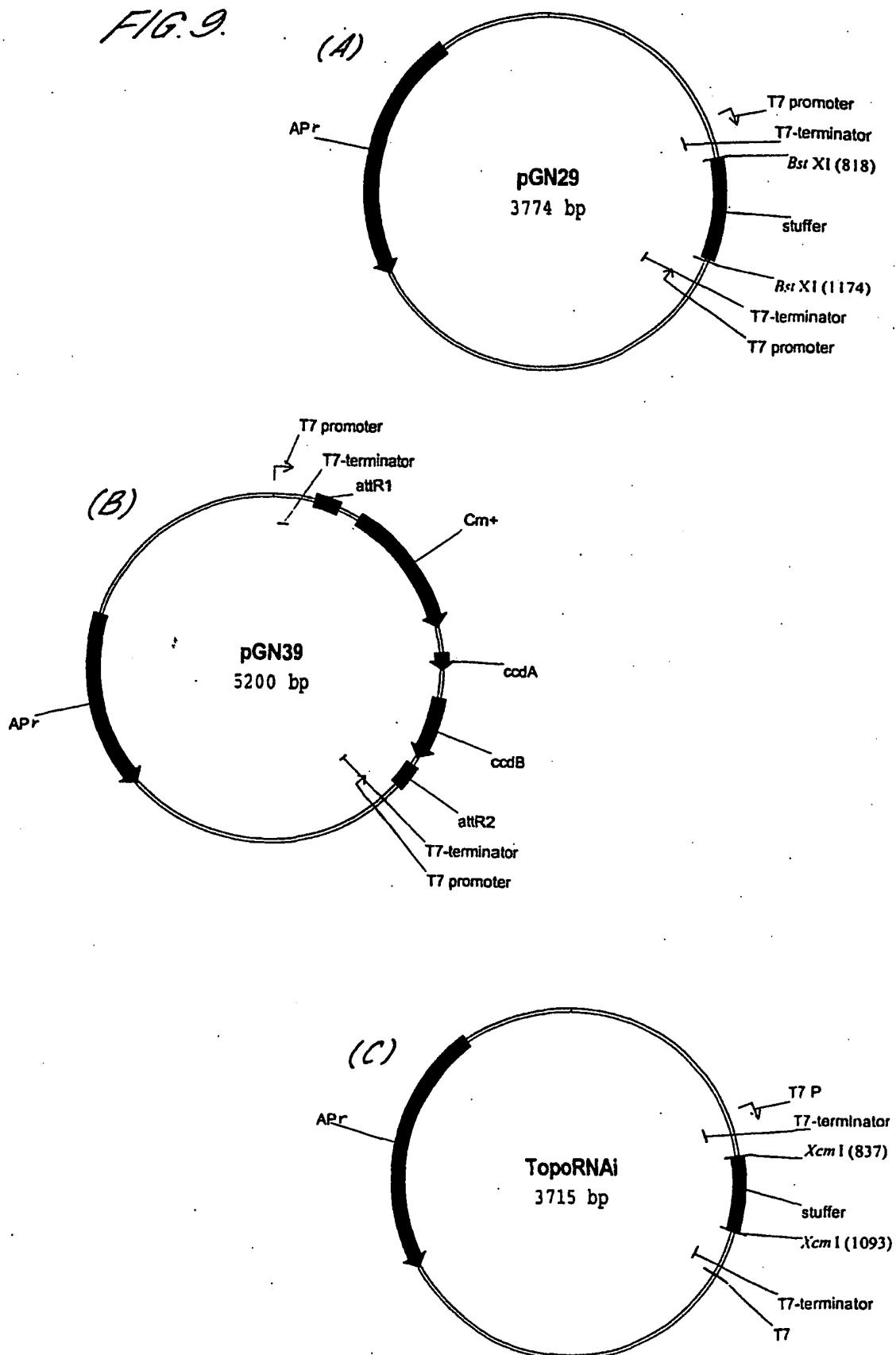


FIG. 10.

PGN9

*FIG. 11.*

PGN29

1 gagtgcacca tatgcgtgt gaaataccgc acagatgcgt aaggagaaaa taccgcacca  
 61 ggcgaaattt taaacgttaa tattttgtta aaattcgcgt taaaatattt ttaaatcagc  
 121 tcattttta accaataaggc cggaaatccgc aaaatccctt ataaatcaaa agaatagacc  
 181 gagatagggt tgagtgttgc tccagtttg aacaagagtc cacttaaa gaacgtggac  
 241 tccaacgtca aaggcgaaaa aaccgttat cagggcgatg gcccactacg tgaaccatca  
 301 cccaaatcaa gtttttgc gtcgaggtgc cgtaaagtc taaaatcgaa ccctaaaggg  
 361 agcccccgat ttagagcttgc acggggaaag cccgcgaacg tggcgagaaa ggaagggaaag  
 421 aaagcgaaag gagcggcgc tagggcgctg gcaagtgtag cggcacgtc ggcgtaaacc  
 481 accacaccog cccgcgttaa tgcgccccta cagggcgatc ccattcgcca ttcaaggctgc  
 541 gcaactgttgc ggaaggcgatc tcgggtgcggg cctcttcgtt attacgcacg ctggcgaaag  
 601 ggggatgtgc tgcaaggcgat ttaagtgtgg taacgcacgg gttttcccgat tcacgcacgtt  
 661 gtaaaacgcg gcccgtgaa ttgtaatacg actcaactata gggcgaattc aaaaaaacc  
 721 tcaagaccgg ttagaggcc ccaagggtt atgctgtatc attctgcagg gtacccgggg  
 781 atccctctaga gatccctcgat cctcgagatc cattgtgtgc ggcggattc ttatcactg  
 841 ataagttgtt ggacatatta tttttatcag tgataaaatgt tcaagcatga caaagtgc  
 901 gccaataaca gtgatccgtg cccgccttgc actgttgcac gaggcggcg tagacggct  
 961 gacgacacgcg aaactggcg aacgggttggg ggtgcacgtc ccggcgctt actggcactt  
 1021 caggaacaacg cgggcgtgc tcgacgcact ggcgcgaagcc atgcgtggcg agaatcatac  
 1081 gcttcgggtgc cgagagccgatc cgacgactgg cgctcattc tgatcgggaa tcccgcacgt  
 1141 tcaggcaggc gctgtcgcc tacggccagc acaatggatc tcgaggatc ttccatcac  
 1201 accagtctgc cgcctcgagg tcgcggccgc gactctctag acgcgtaaac ttactagcat  
 1261 aacccttgg ggcctctaaa cgggtcttgc ggggtttttt gagcttctcg ccctataatg  
 1321 agtctgttata cagttgtatc attctataatc gtcaccaaaa tagcttggcg taatcatatgt  
 1381 catagcttttgc tcctgtgtgc aattttttatc cgctcacaat tccacacaac atacgaggcg  
 1441 gaagcataaa gtgttaagcc tgggtgcctt aatgagttgc ctaactcaca ttaatttgcgt  
 1501 tgcgcgtact gcccgcgtt cagtcgggaa acctgtcgatc ccagctgcat taatgaatcg  
 1561 gccaacgcgcg ggggagggc gtttgcgtt ttggcgctc ttccgccttcc tcgctactg  
 1621 actcgctgcg ctgggtcgatc cgctcgccg gacgggtatc agtcactca aaggcggtaa  
 1681 tacgggttattc cacagaatca gggataacgc cagggaaagaa catgtgagca aaaggccagc  
 1741 aaaaggccag gaaccgtaaa aaggccgcgt tgctggcgat tttcgatagg ctccgcccc  
 1801 ctgacgagca tcacaaaaat ctagctcaatc gtcagaggtg gcggaaacc  
 1861 aaagataccat ggcgttccgc cctggaaatgc ccctcgatc ctctctcg acaggactat  
 1921 cgcttaccggg atacctgtcc gcttttctcc ctggggaaatgc ctggcgctt ttcataatg  
 1981 cacgtgtatc gtatctcgat tcgggttgcgatc tcgatcgatc caagctggc tttgtgcacg  
 2041 aacccttccgt tcaagccgcg cgtcgccctt tatttttttgc tatttttttgc gatgttcc  
 2101 cggtaagaca cgacttacgc ccaactggcag cagccactgg taacaggatt agcagagcg  
 2161 ggtatgttagg cgggtctaca gagtttttgc gatgggtggc taactacggc tacactagaa  
 2221 ggacagtattt tggtatctgc gctctgtgc gtcctgtatc cttcgaaaaa agagttgtt  
 2281 gctcttgcgtt cggcaacaa accaccgcgtt gtagcgggtt ttttttttttgc tgcaagc  
 2341 agattacgcg cagaaaaaaa ggtatctcaatc aagatcttgc ttttttttttgc acgggtct  
 2401 acgtctgttgc gaaacaaaaatc tcaatgttgc ggttttttttgc ttttttttttgc tcaaaa  
 2461 ttttgcgttgc gatcccttta aattttttatc gatgttttgc ttttttttttgc agtataat  
 2521 agttaacttgc gtctgacgtt taccaatgtc taatctgtgc ggcacccatc tcagcgatct  
 2581 gtcttatttgc ttcatccata gttgcctgc tccccgtcgt gtagataact acgataac  
 2641 agggcttaccat atctggccccc agtctgtgc tgcacccgcg agacccacgc  
 2701 cagattttatc agcaataaac cagccagccg gaaggccgc ggcacccatc tcaccggc  
 2761 cttttatccgc ctccatccatc totattttatc gttggggaaatgc agttagatgc  
 2821 cagttatatttgc ttgcgttgc gttgttgc gttgttgc ttttttttttgc tcacgc  
 2881 cgttttttttgc ggcttcattc agtcccgatc cccaaatgc ttttttttttgc acatgatcc  
 2941 ccatgttgc gaaaaaaatgc gttgttgc ttttttttttgc ttttttttttgc agaagtaat  
 3001 tggccgcgtt gttatctgc atgggttgc gacccactgc taatttttttgc actgtcatgc  
 3061 catccgtaaatgc atgcttttgc gttgttgc gttgttgc ttttttttttgc tgagaatacc  
 3121 ggcggccggc accgagttgc ttttgcggc gttgttgc ttttttttttgc gtatgacata  
 3181 gcaagaactt ttttttttttgc ttttttttttgc ttttttttttgc ctctcaagga  
 3241 ttttttttttgc ttttttttttgc ttttttttttgc ttttttttttgc tgatcttgc  
 3301 catcttttgc ttttttttttgc ttttttttttgc ttttttttttgc aatgccca  
 3361 aaaaggaaatgc aaggccgatc cggaaatgtt gatactcat actcttc  
 3421 attgtatc ttttttttttgc ttttttttttgc ttttttttttgc ttttttttttgc tttttttt  
 3481 aaaataaaaca aatagggtt cccgcgcacat ttttttttttgc ttttttttttgc gacgtct  
 3541 aaaccattat tatcatgaca ttttttttttgc ttttttttttgc ttttttttttgc  
 3601 tggcgctt cgggtatgc ggtggaaacc ttttttttttgc ttttttttttgc gacgtcc  
 3661 cagttgtct ttttttttttgc ttttttttttgc ttttttttttgc ttttttttttgc  
 3721 ttggcggtt ttttttttttgc ttttttttttgc ttttttttttgc ttttttttttgc

*FIG. 12.**PGN39*

TAATACGACT CACTATAGGG CGAATTCAAA AAACCCCTCA AGACCCGTTT  
AGAGGCCCA AGGGGTTATG CTAGTGAATT CTGCAGCGGT ACCCGGGGAT  
CCTCTAGAGA TCCCTCGACC TCGAGATCCA TTGTGCTGGA AAGATCACAA  
GTTTGTACAA AAAAGCTGAA CGAGAAACGT AAAATGATAT AAATATCAAT  
ATATTAAATT AGATTTTGCA TAAAAAACAG ACTACATAAT ACTGTAAAAC  
ACAACATATC CAGTCACTAT GGCGGCCGCA TTAGGGACCC CAGGCTTTAC  
ACTTTATGCT TCCGGCTCGT ATAATGTGTG GATTTGAGT TAGGATCCGG  
CGAGATTTTC AGGAGCTAAG GAAGCTAAA TGGAGAAAAA AATCACTGGA  
TATACCACCG TTGATATATC CCAATGGCAT CGTAAAGAAC ATTTTGAGGC  
ATTTCACTCA GTTGCCTCAAT GTACCTATAA CCAGACCGTT CAGCTGGATA  
TTACGGCCTT TTTAAAGACC GTAAAGAAAA ATAAGCACAA GTTTTATCCG  
GCCTTTATTC ACATTCTTGC CCGCCTGATG AATGCTCATC CGGAATTCCG  
TATGGCAATG AAAGACGGTG AGCTGGTGAT ATGGGATAGT GTTCACCCCTT  
GTTACACCGT TTTCCATGAG CAAACTGAAA CGTTTCATC GCTCTGGAGT  
GAATACCACG ACGATTTCCG GCAGTTCTA CACATATATT CGCAAGATGT  
GGCGTGTAC GGTGAAAACC TGGCCTATT CCCTAAAGGG TTATTGAGA  
ATATGTTTT CGTCTCAGCC AATCCCTGGG TGAGTTTCAC CAGTTTGAT  
TTAAACGTGG CCAATATGGA CAACTTCTC GCCCCCGTT TCACCATGGG  
CAAATATTAT ACGCAAGCGC ACAAGGTGCT GATGCCGCTG GCGATTCAAG  
TTCATCATGC CGTCGTGAT GGCTTCCATG TCGGCAGAAAT GCTTAATGAA  
TTACAACAGT ACTGCGATGA GTGGCAGGGC GGGGCGTAAA GATCTGGATC  
CGGCTTACTA AAAGCCAGAT AACAGTATGC GTATTGCGC GCTGATTTTT  
GCGGTATAAG AATATATACT GATATGTATA CCCGAAGTAT GTCAAAAAGA  
GGTGTGCTAT GAAGCAGCGT ATTACAGTGA CAGTTGACAG CGACAGCTAT  
CAGTTGCTCA AGGCATATAT GATGTCAATA TCTCCGGTCT GGTAAAGCACA  
ACCATGCAGA ATGAAGCCCG TCGTCTGCGT GCCGAACGCT GGAAAGCGGA  
AAATCAGGAA GGGATGGCTG AGGTCGCCCG GTTTATTGAA ATGAACGGCT  
CTTTTGCTGA CGAGAACAGG GACTGGTGAA ATGCAGTTA AGGTTTACAC  
CTATAAAAGA GAGAGCCGTT ATCGTCTGTT TGTGGATGTA CAGAGTGATA  
TTATTGACAC GCCCGGGCGA CGGATGGTGA TCCCCCTGGC CAGTGCACGT  
CTGCTGTCAG ATAAAGTCTC CCGTGAACCTT TACCCGGTGG TGCATATCGG  
GGATGAAAGC TGGCGCATGA TGACCACCGA TATGGCCAGT GTGCCGGTCT  
CCGTTATCGG GGAAGAAGTG GCTGATCTA GCCACCGCGA AAATGACATC  
AAAAACGCCA TTAACCTGAT GTTCTGGGA ATATAATGT CAGGCTCCCT  
TATACACAGC CAGTCTGCAG GTCGACCATA GTGACTGGAT ATGTTGTGTT  
TTACAGTATT ATGTAATGCTG TTTTTATGC AAAATCTAAT TTAATATATT  
GATATTATA TCATTTACG TTTCTGTTTC AGCTTCTTG TACAAAGTGG  
TGATCTTCC AGCACAAATGG ATCTCGAGGG ATCTTCCATA CCTACCAGTT  
CTGCGCTGCA AGGTGCGGGC CGCGACTCTA GACGCGTAAG CTTACTAGCA  
TAACCCCTTG GGGCTCTAA ACGGGTCTTG AGGGGTTTT TGAGCTTCTC  
GCCCTATAGT GAGTCGTATT ACAGCTTGAG TATTCTATAG TGTCACCTAA  
ATAGCTTGGC GTAATCATGG TCATAGCTGT TTCTGTGTT AAATTGTTAT  
CCGCTCACAA TTCCACACAA CATAACGAGCC GGAAGCATAA AGTGTAAAGC

*FIG. 12 (CONTINUED 1)*

CTGGGGTGCC TAATGAGTGA GCTAACTCAC ATTAATTGCG TTGCGCTCAC  
TGCCCCCTTT CCAGTCGGGA AACCTGTCGT GCCAGCTGCA TTAATGAATC  
GGCCAACGCG CGGGGAGAGG CGGTTGCGT ATTGGCGCT CTTCCGCTTC  
CTCGCTCACT GACTCGCTGC GCTCGTGT CGGCTGCGG CGAGCGGTAT  
CAGCTCACTC AAAGGCGGTAA ATACGGTTAT CCACAGAATC AGGGGATAAC  
GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA  
AAAGGCCGCG TTGCTGGCGT TTTTCGATAG GCTCCGCCCC CCTGACGAGC  
ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA  
TAAAGATAACC AGGCCTTTCC CCCTGGAAGC TCCCTCGTGC GCTCTCCGT  
TCCGACCCCTG CCGCTTACCG GATAACCTGTC CGCCCTTCTC CCTTCGGAA  
GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG TTCGGTAG  
GTCGTTGCGT CCAAGCTGGG CTGTTGTCAC GAACCCCCCG TTCAGCCGA  
CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGGTAAGAC  
ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT TAGCAGAGCG  
AGGTATGTAG GCGGTGCTAC AGAGTTCTG AAGTGGTGGC CTAACTACGG  
CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA  
CCTTCGGAAA AAGAGTTGGT AGCTCTTGT CGGCAAACA AACCAACCGCT  
GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAA  
AGGATCTCAA GAAGATCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT  
GGAACGAAAA CTCACGTTAA GGGATTTGG TCATGAGATT ATCAAAAAGG  
ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTA AATCAATCTA  
AAAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG  
AGGCACCTAT CTCAGCGATC TGTCTATTTTC GTTCATCCAT AGTTGCCGTA  
CTCCCCGCTG TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC  
CAGTGCCTGCA ATGATACCCG GAGACCCACG CTCACCGGCT CCAGATTTAT  
CAGCAATAAA CCAGCCAGCC GGAAGGGCCG AGCGCAGAACAG TGGTCCTGCA  
ACTTTATCCG CCTCCATCCA GTCTATTAAT TGGTGGCGGG AAGCTAGAGT  
AAAGTAGTTGCG CCAGTTAATA GTTGGCGCAA CGTTGTTGGC ATTGCTACAG  
GCATCGTGGT GTCACGCTCG TCGTTGGTA TGGCTTCATT CAGCTCCGGT  
TCCCAACGAT CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAGC  
GGTTAGCTCC TTGGTGGCTC CGATCGTTGT CAGAAGTAAG TTGGCCCGAG  
TGGTATCACT CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCATG  
CCATCCGTAAT GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT  
CTGAGAAATAC CGCGCCCGGC GACCGAGTTG CTCTGCCCCG GCGTCATAAC  
GGGATAATAG TGTATGACAT AGCAGAACCT TAAAAGTGCT CATCATTGGA  
AAACGTTCTT CGGGGCAGAA ACTCTCAAGG ATCTTACCGC TGTTGAGATC  
CAGTTGATG TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA  
CTTTCACCAAG CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA  
AAAAAGGGAA TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT  
TTTCAATAT TATTGAAGCA TTTATCAGGG TTATTGTCCT ATGAGCGGAT  
ACATATTTGA ATGTATTTAG AAAATAAAC AAATAGGGT TCCGCGCACA  
TTTCCCCGAA AAGTGGCACC TGACGTCATA GAAACCATTAA TTATCATGAC  
ATTAACCTAT AAAATAAGGC GTATCAGGAG GCCCTTTCGT CTCGCGCGTT  
TCGGTGATGA CGGTGAAAC CTCTGACACA TGCAGCTCCC GGAGACGGTC  
ACAGCTGTC TGTAGCGGA TGCCGGGAGC AGACAAGCCC GTCAGGGCGC  
GTCAGCGGGT GTTGGCGGGT GTCGGGGCTG GCTTAACCTAT CGGGCATCAG

*FIG. 12 (CONTINUED 2)*

AGCAGATTGT ACTGAGAGTG CACCATATGC GGTGTGAAAT ACCGCACAGA  
TGCCTAAGGA GAAAATACCG CATCAGGCGA AATTGTAACG GTTAATATTT  
TGTAAATT CGCGTTAAAT ATTTGTTAAA TCAGCTCATT TTTAACCAA  
TAGGCCGAAA TCGGAAAAT CCCTTATAAA TCAAAAGAAT AGACCGAGAT  
AGGGTTGAGT GTTGTCCAG TTTGGAACAA GAGTCCACTA TTAAAGAACG  
TGGACTCCAA CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA  
CTACGTGAAC CATCACCCAA ATCAAGTTTT TTGCGGTGCA GGTGCCGTAA  
AGCTCTAAAT CGGAACCTA AAGGGAGCCC CCGATTAGA GCTTGACGGG  
GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC GAAAGGAGCG  
GGCGCTAGGG CGCTGGCAAG TGTAGCGTC ACCTGCGCG TAACCACCAC  
ACCCGCCGCG CTAAATGCGC CGCTACAGGG CGCGTCCATT CGCCATTCA  
GCTGCCAAC TGTTGGGAAG GGCGATCGGT GCGGGCCTCT TCGCTATTAC  
GCCAGCTGGC GAAAGGGGGA TGTGCTGCAA GGCGATTAAG TTGGGTAACG  
CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACGGCCA GTGAATTG

*FIG. 13.*

TopoRNAi

1 gagtgcacca tatgcggtgt gaaataccgc acagatgcgt aaggagaaaa taccgcata  
 61 ggcgaaattt taaaacgttaa tattttgtta aaattcgcgt taaaatatttgc taaaatcagc  
 121 tcattttta accaataggc cgaaatcgcc aaaatcccctt ataaatcaaa agaatagacc  
 181 gagatagggt tgagtgttgc tccaggttgg aacaagagtc cactattaaa gaacgtggac  
 241 tccaaacgtca aaggcgaaa aaccgtctat cagggcgatg gccactacg tgaaccatca  
 301 cccaaatcaa gtttttgcg gtgcagggtgc cgtaaagctc taaaatcgaa ccctaagg  
 361 agcccccgat tttagagcttgc acggggaaag ccggcgaaacg tggcgagaaa ggaagggaag  
 421 aaagcgaaag gaggcgccg tagggcgctg gcaagtgttag cggtcacgct ggcgtaaacc  
 481 accacaccccg cccgcgtttaa tgcgcgcgtc cagggcgctg ccattcgcca ttcatcgctc  
 541 gcaactgttgc ggaaggcgca tcgggtcgccc ccttcgtcgtt attacgcacg ctggcgaaag  
 601 ggggatgtgc tgcaaggcgca ttaagttggg taacggccagg gttttcccg tcacgcgtt  
 661 gtaaaacgcg ggcgcgttgc ttgtatatacg actcactatc ggccgaaattc aaaaaacccc  
 721 tcaagaccccg ttagaggcc ccaagggttgc atgctgtatc attctgcagg gtacccgggg  
 781 atcctctaga gatccctcgat cctcgagatc cattgtgggtt gatttctacc aaggctagca  
 841 tggcagccg aatacagtga tccgtccggg ccctggactt ttaaacgagg tcggcgtaga  
 901 cggctctgacg acacgcaaaac tggcgaaacg gttgggggtt cggcagccgg cgctttactg  
 961 gcacttcagg aacaagcggg cgctgtcgat cgcactggcc gaagccatgc tggcgagaa  
 1021 tcatacgctt cgggtccggg agccgcacgacttgcgt catttctgtatc cgggaatccc  
 1081 gcagccatgc tagcttgcgtt gaaattccac cacaatggat tcggaggat ctccatata  
 1141 taccagtttgc ggcgcgtcgg gtcgcggcccg cgactctctatc gacgcgttaag cttaactagca  
 1201 taacccttgc gggcctctaa acgggtcttgc aggggttttt tgagtttctc gcccstatatg  
 1261 gagtcgtatt acagcttgat tattctatag tgcacccatc atagcttggc gtaatcatgg  
 1321 tcatacgctt ttcctgtgtt aatttgcgtt cgcgtccatc ttccacacaa catacgagcc  
 1381 ggaagcataa agtgttgcgtt ctgggggttgcg taatgtgtt gctaactcactt aataatttgcg  
 1441 ttgcgtcact tgcccgttcc ccaatgttgcgtt ggcggccgtt ctcgcgttactc gtcgcgtact  
 1501 ggcqaaacgcg cggggagagg cgggttgcgtt atggggcgat ctcgcgttactc aaaggcggt  
 1561 gactcgctgc gtcgggtcgat tccgtcgccg cggccgttat cgcgttactc  
 1621 atacgggttat ccacagaatc aggggataac gcaggaaaaga acatgtgago aaaaggccag  
 1681 caaaaggccca ggaaccgtaa aaggccgcg ttgcggcgat tttcgatag gctccggccc  
 1741 cctgacgacg atcacaaaaaa tcgacgtcacta agtcagatgg ggcgaaaccc gacaggacta  
 1801 taaagatacc aggcttcc cccgttgcgtt tccctcgatc gctctctgt tccgacccctg  
 1861 ccgcttacccg gatacctgtc cgccttctc ccttgcggaa gcttgcgtt ttcgcgtact  
 1921 tcacgtctgtt ggtatctcgat tccgttgcgtt gtcgttgcgtt ccaagctggg ctgtgtgcac  
 1981 gaaccccccgtt ttcagcccgat cccgtcgccg tttatccgttactatcgatc tgagtccaa  
 2041 ccggttacccg acgacttacc ggcactggca gcagccactt gtaacaggat tagcagagcg  
 2101 aggtatgtatc gccgttgcgtt aggttgcgtt aagtgggtgc ctaactacgg ctacactaga  
 2161 aggacagttat ttggatctgt cgcctctgtt aagccagttt ctttcggaaa aagagttgg  
 2221 agcttgcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt ttgcaagcag  
 2281 cagattacgc gcagaaaaaa aggttgcgtt gaaatgttccat tgcgttgcgtt tacggggctt  
 2341 gacgcttgcgtt ggaacaaaaaa ctcacgtttaa gggatttttttgcgtt tgcgttgcgtt  
 2401 atcttcacctt agatccctttt aatattaaaaaa tgaatgttta aatcaatctt aatctatata  
 2461 gagtaaactt ggttgcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 2521 tgtcttgcgttccat gtttgcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 2581 gagggcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 2641 ccagatccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 2701 actttatccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 2761 ccagatccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 2821 tcgttgcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 2881 cccatgttgcgtt gcaaaaaaa ggttgcgttccat cccggaaacaa aaccaccgcgtt gttttttgtt  
 2941 ttggccgcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3001 ccatccgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3061 cgcgccccggc gaccggatggtccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3121 agcagaactt taaaatgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3181 atcttcacctt cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3241 gcatccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3301 aaaaaggggaa taaggcgac acggggatggtccat cccggaaacaa aaccaccgcgtt gttttttgtt  
 3361 tatttgcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3421 aaaaataaaac aaatagggttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3481 gaaaccatccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3541 ctcgcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3601 acagcttgcgttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt  
 3661 gttggcggttccat cccggaaacaa aaccaccgcgtt ggttgcgtt gttttttgtt

*PGN49A**FIG. 14.*

TGTAATACGA CTCACTATAG GGCGAATTCA AAAAACCCCT CAAGACCCGT  
TTAGAGGCC CAAGGGGTTA TGCTAGTGAA TTCTGCAGCG GTACCCGGGG  
ATCCTCTAGA GATCCCTCGA CCTCGAGATC CATTGTGCTG GAAAGGATCT  
GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT TGCGCGCTGA  
TTTTTGCGGT ATAAGAATAT ATACTGATAT GTATACCGA AGTATGTCAA  
AAAGAGGTGT GCTATGAAGC AGCGTATTAC AGTGACAGTT GACAGCGACA  
GCTATCAGTT GCTCAAGGCA TATATGATGT CAATATCTCC GGTCTGGTAA  
GCACAACCAC GCAGAAATGAA GCCCCGTCGTC TGCGTGCCGA ACGCTGGAAA  
GCGGAAAATC AGGAAGGGAT GGCTGAGGTC GCCC GGTTA TTGAAATGAA  
CGGCTCTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA GTTTAAGGTT  
TACACCTATA AAAGAGAGAG CGGTTATCGT CTGTTTGTTG ATGTACAGAG  
TGATATTATT GACACGCCCG GGCGACGGAT GGTGATCCCC CTGGCCAGTG  
CACGTCTCTT AAGCGATAAA GTCTCCCGTG AACTTTACCC GGTGGTGCAT  
ATCGGGGATG AAAGCTGGCG CATGATGACC ACCGATATGG CCAGTGTGCC  
GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC CGCGAAAATG  
ACATCAAAAA CGCCATTAAC CTGATGTTCT GGGGAATATA AATGTCAGGC  
TCCCTTATAC ACAGCCTTTC CAGCACAATG GATCTCGAGG GATCTTCCAT  
ACCTACCAGT TCTGCGCCTG CAGGTCGCGG CCGCGACTCT AGACCGTAA  
GCTTACTAGC ATAACCCCTT GGGGCCTCTA AACGGGTCTT GAGGGGTTTT  
TTGAGCTTCT CGCCCTATAG TGAGTCGTAT TACAGCTTGA GTATTCTATA  
GTGTCACCTA AATAGCTTGG CGTAATCATG GTCATAGCTG TTTCTGTGT  
GAAATTGTTA TCCGCTCACA ATTCCACACA ACATACGAGC CGGAAGCATA  
AAGTGTAAAG CCTGGGGTGC CTAATGAGTG AGCTAACTCA CATTAAATTGC  
GTTGCGCTCA CTGCCCCTT TCCAGTCGGG AAACCTGTCG TGCCAGCTGC  
ATTAATGAAT CGGCCAACGC GCGGGGAGAG GCGGTTTGCG TATTGGGCGC  
TCTTCCGCTT CCTCGCTCAC TGACTCGCTG CGCTCGGTG 1TCCGCTGCG  
GCGAGCGGT A TCAGCTCACT CAAAGGGGT AATACGGITA TCCACAGAAT  
CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC  
AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTCGATA GGCTCCGCC  
CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC  
CGACAGGACT ATAAAGATAAC CAGGCCTTTC CCCCTGGAAG CTCCCTCGTG  
CGCTCTCCGT TTCCGACCCCT GCGCCTTACG GGATACCTGT CCGCCCTTCT  
CCCTCGGGGA AGCGTGGCGC TTTCTCATAG CTCACGCTGT AGGTATCTCA  
GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC  
GTTCAGCCCG ACCCGTGCAC CTTATCCGT AACTATCGTC TTGAGTCCAA  
CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA  
TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTCCTT GAAGTGGTGG  
CCTAACTACG GCTACACTAG AAGGACAGTA TTTGGTATCT GCGCTCTGCT  
GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTGAA TCCGGCAAAC  
AAACCACCGC TGGTAGCGGT GGTTTTTTG TTTGCAAGCA GCAGATTACG  
CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC  
TGACGCTCAG TGGAAACGAAA ACTCACGTTA AGGGATTGG GTCATGAGAT  
TATCAAAAAG GATTTCAAC TAGATCCTTT TAAATTAAGA ATGAAGTTT

*FIG. 14 (CONTINUED)*

AAATCAATCT AAAGTATATA TGAGTAAACT TGGCTTGACA GTTACCAATG  
CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTG CGTTCATCCA  
TAGTTGCCG ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA  
CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC GCTCACCGGC  
TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA  
GTGGTCTGTC AACCTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG  
GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCAGCA ACGTTGTTGG  
CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTGGT ATGGCTTCAT  
TCAGCTCCGG TTCCCAACGA TCAAGGCAGG TTACATGATC CCCCATGTTG  
TGCAAAAAAG CGGTTAGCTC CTTCCGGCCT CCGATCGTTG TCAGAAGTAA  
GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC  
TTACTGTCAT GCCATCCGTA AGATGCTTT CTGTGACTGG TGAGTACTCA  
ACCAAGTCAT TCTGAGAATA CCGCGCCCG CGACCGAGTT GCTCTTGCCC  
GGCGTCAAATA CGGGATAATA GTGTATGACA TAGCAGAACT TTAAAAGTGC  
TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG  
CTGTTGAGAT CGAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC  
AGCATCTTT ACTTTCACCA GCGTTCTGG GTGAGCAAAA ACAGGAAGGC  
AAAATGCCGC AAAAAGGGG ATAAGGGCGA CACGGAAATG TTGAATACTC  
ATACTCTTCC TTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT  
CATGAGCGGA TACATATTG AATGTATTAA GAAAAATAAA CAAATAGGGG  
TTCCGCGCAC ATTTCGGCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT  
ATTATCATGA CATTAAACCTA TAAAAATAGG CGTATCACGA GGCCCTTTCG  
TCTCGCCGT TTCCGTGATG ACGGTGAAAA CCTCTGACAC ATGCAGCTCC  
CGGAGACGGT CACAGCTTGT CTGTAAGCGG ATGCCGGGAG CAGACAAGCC  
CGTCAGGGCG CGTCAGCGGG TGTGGCGGG TGTGGGGCT GGCTTAACTA  
TGCAGGCACTA GAGCAGATTG TACTGAGAGT GCACCATATG CGGTGTGAAA  
TACCGCACAG ATGCGTAAGG AGAAAATACC GCATCAGGCG AAATTGTA  
CGTTAATATT TTGTTAAAAT TCGCGTTAAA TATTGTTAA ATCAGCTCAT  
TTTTAACCA ATAGGCCGA ATCGGCAAAA TCCCTTATAA ATCAAAAGAA  
TAGACCGAGA TAGGGTGTGAG TGTGTTCCA GTTGGAAACA AGAGTCCACT  
ATTAAAGAAC GTGGACTCCA ACGTCAAAGG GCGAAAAACC GTCTATCAGG  
GCGATGGCCC ACTACGTGAA CCATCACCCA AATCAAGTTT TTTGCGGTCC  
AGGTGCGTA AAGCTCTAAA TCGGAACCCCT AAAGGGAGCC CCCGATTTAG  
AGCTTGACGG GGAAAGCCGG CGAACGTGGC GAGAAAGGAA GGGAAAGAAAG  
CGAAAGGAGC GGGCGCTAGG GCGCTGGCAA GTGTAGCGGT CACGCTGCGC  
GTAACCACCA CACCCGCCGC GCTTAATGCG CCGCTACAGG GCGCGTCCAT  
TCGCCATTCA GGCTGCGCAA CTGTTGGAA GGGCGATCGG TGCAGGGCCTC  
TTCGCTATTA CGCCAGCTGG CGAAAGGGGG ATGTGCTGCA AGGCGATTAA  
GTTGGGTAAC GCCAGGGTTT TCCCAGTCAC GACGTTGAA AACGACGGCC  
AGTGAAT

*PGN59A FIG. 15.*

GAGTGCACCA TATGCGGTGT GAAATACCGC ACAGATGCGT AAGGAGAAAA  
TACCGCATCA GGCAGAATTG TAAACGTTAA TATTTTGTAA AAATTCGCGT  
TAAATATTG TTAAATCAGC TCATTTTTA ACCAATAGGC CGAAATCGGC  
AAAATCCCTT ATAAATCAA AGAATAGACC GAGATAGGGT TGAGTGTGTTG  
TCCAGTTGG AACAAAGAGTC CACTATTAAA GAACGTGGAC TCCAACGTCA  
AAGGGCGAAA AACCGTCTAT CAGGGCGATG GCCCACTACG TGAACCATCA  
CCCAAATCAA GTTTTTGCG GTCGAGGTGC CGTAAAGCTC TAAATCGGAA  
CCCTAAAGGG AGCCCCCGAT TTAGAGCTTG ACGGGGAAAG CCGGCGAACG  
TGGCGAGAAA GGAAGGGAAG AAAGCGAAAG GAGCGGGCGC TAGGGCGCTG  
GCAAGTGTAG CGGTACGCT GCGCGTAACC ACCACACCCG CCGCGCTTAA  
TGCGCCGCTA CAGGGCGCGT CCATTCGCCA TTCAGGCTGC GCAACTGTTG  
GGAAGGGCGA TCGGTGCGGG CCTCTTCGCT ATTACGCCAG CTGGCGAAAG  
GGGGATGTGC TGCAAGGCGA TTAAGITGGG TAACGCCAGG GTTTTCCCAG  
TCACGACGTT GTAAAACGAC GGCGAGTGAA TTGTAATACG ACTCACTATA  
GGGCGAATTC GAGCTCGGT CCCGGGGATC CTCTAGAGAT CCCTCGACCT  
CGAGATCCAT TGTGCTGGAA AGGATCTGGA TCCGGCTTAC TAAAAGCCAG  
ATAACAGTAT GCGTATTGTC GCGCTGATTT TTGCGGTATA AGAATATATA  
CTGATATGTA TACCCGAAGT ATGTCAAAAA GAGGTGTGCT ATGAAGCAGC  
GTATTACAGT GACAGTTGAC AGCGACAGCT ATCAGTTGCT CAAGGCATAT  
ATGATGTCAA TATCTCCGGT CTGGTAAGCA CAACCATGCA GAATGAAGCC  
CGTCGTCTGC GTGCCGAACG CTGAAAGCG GAAAATCAGG AAGGGATGGC  
TGAGGTCGCC CGGTTTATTG AAATGAACGG CTCTTTTGCT GACGAGAAC  
GGGACTGGTG AAATGCAGTT TAAGGTTTAC ACCTATAAAA GAGAGAGCCG  
TTATCGTCTG TTTGTGGATG TACAGAGTGA TATTATTGAC ACGCCCGGGC  
GACGGATGGT GATCCCCCTG GCCAGTGCAC GTCTCTTAAG CGATAAAAGTC  
TCCCGTGAAC TTTACCCGGT GGTGCATATC GGGGATGAAA GCTGGCGCAT  
GATGACCACC GATATGCCA GTGTGCCGGT CTCCGTTATC GGGGAAGAAG  
TGGCTGATCT CAGCCACCGC GAAAATGACA TCAAAAACGC CATTAACTCG  
ATGTTCTGGG GAATATAAAT GTCAGGCTCC CTTATACACA GCCTTCCAG  
ACAATGGAT CTCGAGGGAT CTTCATACC TACCAGTTCT GCGCCTGCAG  
GTCGGGGCC CGACTCTCTA GAGTCGAAAG CTTCTCGCC TATAGTGACT  
CGTATTACAG CTTGAGTATT CTATAGTGTG ACCTAAATAG CTTGGCGTAA  
TCATGGTCAT AGCTGTTTCC TGTGTGAAAT TGTTATCCGC TCACAATTCC  
ACACAACATA CGAGCCGGAA GCATAAAAGTG TAAAGCCTGG GGTGCCTAAT  
GAGTGAGCTA ACTCACATTA ATTGCGTTGC GCTCACTGCC CGCTTCCAG  
TCGGGAAACC TGTCGTGCCA GCTGCATTA TGAATCGGCC AACGCGCGGG  
GAGAGGCGGT TTGCGTATTG GGCCTCTTC CGCTTCCTCG CTCACTGACT  
CGCTCGCCTC GGTGCTTCGG CTGCCGGAG CGGTATCAGC TCACTCAAAG  
GCCGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCGAG GAAAGAACAT  
GTGAGCAAA GGCCAGCAA AGGCCAGGAA CGCTAAAAG GCCGCGTTGC  
TGGCGTTTTT CGATAGGCTC CGCCCCCTG ACGAGCATA CAAAAATCGA  
CGCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA GATACCAGGC  
GTTTCCCCCT GGAAGCTCCC TCGTGCCTC TCCGTGTTCCG ACCCTGCCGC  
TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT

*FIG. 15 (CONTINUED)*

CATAGCTCAC GCTGTAGGTA TCTCAGITCG GTGTAGGTCG TTCGCTCCAA  
GCTGGGCTGT GTGCACGAAC CCCCCGTTCA GCCCGACCGC TGCGCCTTAT  
CCGGTAACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA CTTATGCCA  
CTGGCAGCAG CCACITGGTAA CAGGATTAGC AGAGCGAGGT ATGTAGGCAG  
TGCTACAGAG TTCTTGAAAGT GGTGGCCTAA CTACGGCTAC ACTAGAAGGA  
CAGTATTGAG TATCTGCGCT CTGCTGAAGC CAGTACCTT CGGAAAAAGA  
GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT  
TTTTGTTGCA AAGCAGCAGA TTACGCGAG AAAAAGGA TCTCAAGAAG  
ATCCCTTGAT CTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAAACCTCA  
CGTTAAGGA TTTTGGTCAT GAGATTATCA AAAAGGATCT TCACCTAGAT  
CCTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT ATATATGAGT  
AAACTGGTC TGACAGTTAC CAATGCTTAA TCAGTGAGGC ACCTATCTCA  
GCGATCTGTC TATTCGTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA  
GATAACTACG ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA  
TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTATCAGC AATAAACAG  
CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT CCTGCAACTT TATCCGCCCTC  
CATCCAGTCT ATTAATTGTT GCGGGAAAGC TAGAGTAAGT AGTTGCCAG  
TTAATAGTTT GCGCAACGTT GTTGGCATTG CTACAGGCAT CGTGGTGTCA  
CGCTCGTCGT TTGGTATGGC TTCATTCAAGC TCCGGTCCC AACGATCAAG  
GCGAGTTACA TGATCCCCA TGTGTCAGA AAAAGCGGTT AGCTCCTTCG  
GTCCTCCGAT CGTTGTCAGA AGTAAGTGG CCGCAGTGGT ATCACTCATG  
GTTATGGCAG CACTGCATAA TTCTCTTACT GTCTGCCAT CCGTAAGATG  
CTTTCTGTG ACTGGTGGAGT ACTCAACCAA GTCAATTCTGA GAATACCGCG  
CCGGCGACC GAGTTGCTCT TGCCCGCGT CAATACGGGA TAATAGTGTAA  
TGACATAGCA GAACCTTAAA AGTGCTCATC ATTGGAAAAGC GTTCTCGGG  
GCGAAAACTC TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC  
CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTACTTT CACCAGCGTT  
TCTGGGTGAG CAAAAACAGG AAGGCAAAT GCGCAAAAA AGGGATAAG  
GGCGACACGG AAATGTTGAA TACTCATACT CTTCTTTT CAATATTATT  
GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATAACAT ATTTGAATGT  
ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT  
GCCACCTGAC GTCTAAGAAA CCATTATTAT CATGACATTA ACCTATAAAA  
ATAGGGCTAT CACGAGGCC CTTCGTCTCG CGCGTTTCGG TGATGACGGT  
GAAAACCTCT GACACATGCA GCTCCCGAG ACGGTCACAG CTTGTCTGTAA  
AGCGGATGCC GGGAGCAGAC AAGCCCGTCA GGGCGCGTCA GCGGGTGTG  
GCGGGTGTG GGGCTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG

A

FIG. 16.

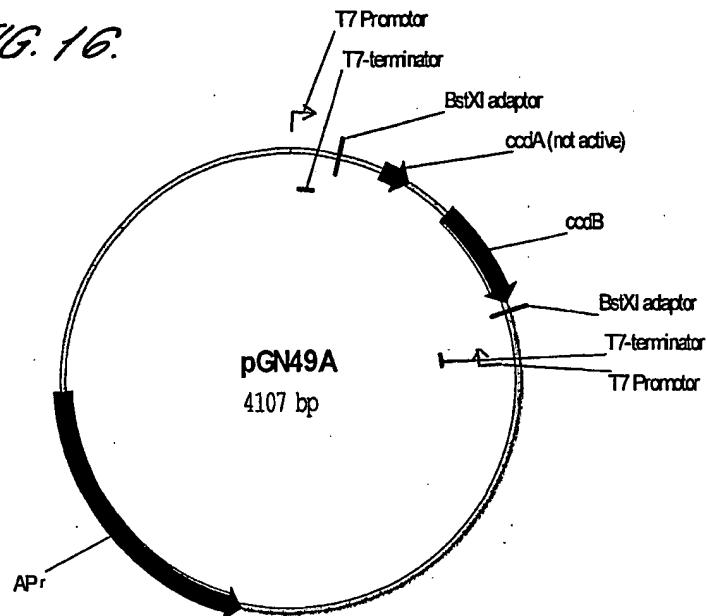
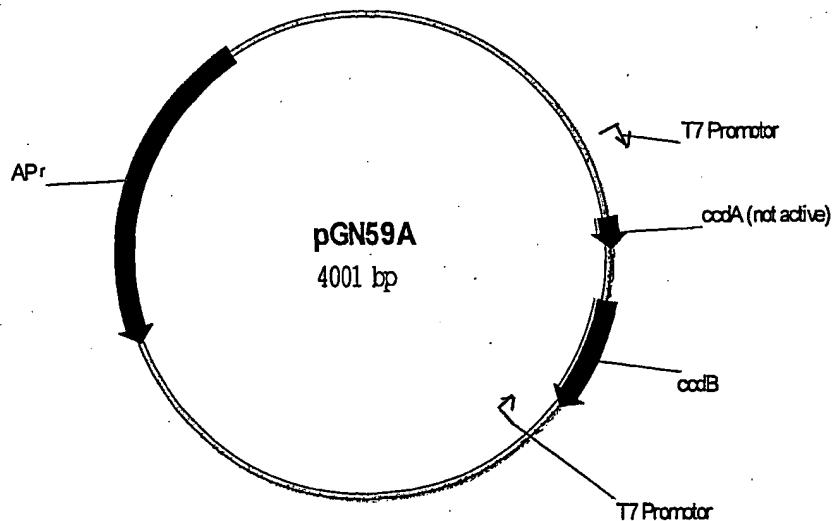


FIG. 17.



## SEQUENCE LISTING

<110> DEVGEM NV

<120> VECTOR CONSTRUCTS

<130> SCB/55178/001

<140>

<141>

<160> 21

<170> PatentIn Ver. 2.0

<210> 1

<211> 160

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Fragment of  
pGN1 containing opposable T7 promoters

<400> 1

ttgttaatacg actcaactata gggcgaattc gagctcggtt cccggggatc ctcttagagtc 60  
gaaagcttct cgccctatag ttagtcgtat tacagcttga gtattctata gtgtcaccta 120  
aatagcttgg cgtaatcatcg gtcatacgctg tttcctgtgt 160

<210> 2

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: DNA sequence  
containing a T7 terminator

<400> 2

actagcataa ccccttgggg cctctaaacg ggtcttgagg ggtttttg

49

<210> 3

<211> 70

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:  
Oligonucleotide oGN27

<400> 3

aattcaaaaa acccctcaag acccgtttag aggcggcaag gggttatgct agtgaattct 60  
gcagcggtac 70

<210> 4

<211> 62

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

## Oligonucleotide oGN28

&lt;400&gt; 4

cgctgcagaa ttcactagca taaccccttg gggcctctaa acgggtcttg aggggaaaa 60  
tg 62

&lt;210&gt; 5

&lt;211&gt; 65

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:  
Oligonucleotide oGN29

&lt;400&gt; 5

ctagacgcgt aagcttacta gcataacccc ttggggcctc taaaacgggtc ttgaggggtt 60  
tttttg 65

&lt;210&gt; 6

&lt;211&gt; 65

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:  
Oligonucleotide oGN30

&lt;400&gt; 6

agctaaaaaa acccctcaag acccgtagg aggccccaaag gggttatgtt agtaagctta 60  
cgcgt 65

&lt;210&gt; 7

&lt;211&gt; 230

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Fragment of  
plasmid pGN9 containing opposable T7 promoters and  
T7 transcription terminators

&lt;400&gt; 7

ttgttaatacg actcaactata gggcgaattc aaaaaacccc tcaagacccg ttttagaggcc 60  
ccaagggggtt atgctagtga attctgcagg gtacccgggg atectctaga cgcgttaagct 120  
tactagcata acccccttggg gcctctaaac gggtcttgag gggtttttg agcttctcgc 180  
cctatagtga gtcgtattac agcttgagta ttctatagtg tcacctaaat 230

&lt;210&gt; 8

&lt;211&gt; 3323

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Plasmid pGN9

&lt;400&gt; 8

gagtgcacca tatgcggtgtt gaaataccgc acagatgcgt aaggagaaaa taccgcata 60  
ggcggaaatgg taaacgttaa tattttgtta aaattcgcgt taaatatttg ttaaatcagc 120  
tcatttttta accaataggc cgaaatcgcc aaaatccctt ataaatcaaa agaatacgacc 180  
gagatagggt tgagtgttgtt ccagtttg aacaagatgc cactattaaa gaacgtggac 240

tccaaacgtca aaggcgaaa aaccgtctat cagggcgatg gcccactacg tgaaccatca 300  
ccccaatcaa gtttttgcg gtcgagggtgc cgtaaagctc taaatcgaa ccctaaaggg 360  
agcccccgat ttagagctt acggggaaag cggcgaacg tggcgagaaa ggaagggaag 420  
aaagcgaaag gagcgccgc tagggcgctg gcaagtgtag cggtacgct ggcgtaaacc 480  
accacacccg ccgcgttta tgccgcgcta cagggcgctg cattcgcca ttcaggtctgc 540  
gcaactgtt ggaaggcgta tcgggtcgaaa cctctcgct attacgcccag ctggcgaaag 600  
ggggatgtgc tgcaaggcgta ttaagttggg taacgccagg gtttcccaag tcacgacggt 660  
gtaaaacgac ggccagtgaa ttgtataacg actcactata gggcgaattc aaaaaacccc 720  
tcaagacccg ttttagaggcc ccaagggggtt atgctagtga attctgcagg gtacccgggg 780  
atccctctaga cgcgtaaagct tactagcata acccccttggg gcctctaaac gggctctgag 840  
gggttttttgc agcttcgcg cctatagtgta gtcgtattac agcttgagta ttctatagt 900  
tcacctaata agcttggcgta aatcatggc atagctgttt cctgtgtgaa attgttatcc 960  
gctcacaatt ccacacaaca tacgagccgg aagcataaaag tgtaaagcct ggggtgccta 1020  
atgagtgagc taactcacat taattgcgtt ggcgtcaactg cccgctttcc agtcggggaaa 1080  
cctgtcgtc cagctgcatt aatgaatcg ccaacgcg gggagagggcg gtttgcgtat 1140  
tgggcgcctc tccgcttcct cgctcaactg ctcgcgtcg tcggcgcttc ggctgcggcg 1200  
agcggatc a gtcactcaa aggccgtaat acggttatcc acagaatcag gggataaacgc 1260  
aggaaagaaac atgtgagcaa aaggccagca aaaggccagg aaccgtaaaa aggccgcgtt 1320  
gctggcggtt ttcatagggc tccggccccc tgacgagcat cacaaaaaatc gacgctcaag 1380  
tcagaggtgg cgaaacccga caggactata aagataccag gcgtttcccc ctggaaagctc 1440  
cctcgtgcgc tctccgttcc cgaccctgccc gtttacccgga tacctgtccg ctttctccc 1500  
ttcgggaaagc gtggcgctt ctcatagctc acgctgtagg tatctcagtt cggtgttaggt 1560  
cgttcgctcc aagctgggct gtgtgcacga acccccccgtt cagcccgacc gctgcgcctt 1620  
atccggtaaac tatcgttttg agtccaaaccc ggtaaagacac gacttatcgc cactggcago 1680  
agccacttgtt aacaggatta gcagagcgag gtatgttaggc ggtgtacag agttctgaa 1740  
gtggggcctt aactacggct acactagaag gacagtattt ggtatctgcg ctctgtgaa 1800  
gccagttacc ttccggaaaaa gagttggtag ctcttgatcc gccaacaaaaa ccaccgcgtt 1860  
tagcgggtgtt ttttttggtaa gcaagcagca gattacgcgc agaaaaaaaaag gatctcaaga 1920  
agatcccttg atctttctt cggggtctga cgctcaagtgg aacgaaaaact cacgttaagg 1980  
gattttggtc atgagattat caaaaaggat cttcacctag atccctttaa attaaaaatg 2040  
aagttttaaa tcaatctaaa gtatatatga gtaaacttgg totgacagtt accaatgttt 2100  
aatcgtgag gacccatct cagcgatctg tctatttctg tcatccatag ttgcctgact 2160  
ccccgtctg tagataacta cgataccggg gggcttacca tctggccccc gtgctcaat 2220  
gataccgcga gaccacgcg caccggctcc agatttatca gcaataaaacc agccagccgg 2280  
aaggcccgag cgccagaatgt gtccgtcaac ttatccggcc tccatccagt ctatattt 2340  
ttgcccggaa gcttagatgtatgttcccg agttaatagt ttgcgaacag ttgttgcatt 2400  
tgctacagggc atcggtgtt cagctcgcc gtttggatg gtttcatca gtcgggttc 2460  
ccaacgatca aggccgatgtt catgatcccc catgttgcg aaaaaagccg tttagctctt 2520  
cggtcctccg atcggtgtca gaagtaggtt ggccgcagtg ttactactca tggttatggc 2580  
agcaactgcatt aattctctt ctgtcatgcc atccgttaaga tgctttctg tgactgtga 2640  
gtactcaacc aagtccatct gagaataccg cgccoggcga ccgagggtct cttgcccggc 2700  
gtcaataccg gataatagt tatgacatag cagaacttta aaagtgcgtca tcattggaaa 2760  
acgttctcg gggcgaaaac tctcaaggat cttaccgctg ttgagatcca gttcgatgt 2820  
acccactcgat gcacccaaact gatcttcgcg atctttact ttccaccagcg tttctgggt 2880  
agcaaaaaca ggaaggcggaa atgcccggaa aaaggaaata agggcgacac gggaaatgtt 2940  
aataactcata ctcttcctt tcaatattaa ttgaagcatt tatcagggtt attgtctcat 3000  
gagccgatcat atatttgaat gtatttagaa aaataaacaat ataggggtt cgcgcacatt 3060  
tccccggaaa gtccacccgt acgtctaaagaa aaccattatt atcatgacat taacctataa 3120  
aaataggcgat atcacgaggc ctttcgtct cgcgcgttcc ggtgtatgacg tgaaaaacct 3180  
ctgacacatg cagctcccg agacgggtcac agcttgcgtg taagccggatg ccggggagcag 3240  
acaagcccgat cagggcgatg cagccgggtt gggccgggtt cggggctggc ttaactatgc 3300  
ggcatcgagcagattgtac tga 3323

<210> 9  
<211> 3774

<212> DNA

<213> Art:

<223>

<400> 9

gatgtgcacca tatgcgggtgt gaaataccgc acagatgcgt aaggagaaaa taccgcacca 60  
ggcgaattt gtaaacgttaa tattttgtta aaattcgctgtaaatatttt ttaaatcagc 120  
tcattttta accaataggc cgaatcgccaaaatccctt ataaatcaaa agaatagacc 180  
gagatagggt tgagtgttgt tccagtttg aacaagagtc cactattaaa gaacgtggac 240  
tccaacgtca aaggcgaaa aaccgtctat cagggcgatg gcccactacg tgaaccatca 300  
cccaaatcaa gtttttgcg gtcgagggtc cgtaaaagctc taaatcgaa ccctaaaggg 360  
agcccccgat ttagagctt acggggaaaag ccggcgaacg tggcgagaaa ggaagggaaag 420  
aaagcgaaaag gagcgggccc tagggcgctg gcaagtgttag cggtcacgct ggcgtaaacc 480  
accacacccg cccgcgttaa tgccgcgtca cagggcgctg ccattcgcca ttcaggctgc 540  
gcaactgtt ggaaggcgaa tcgggtcggt cctcttcgtt attacgcccag ctggcgaaag 600  
ggggatgtgc tgcaaggcgaa ttaagttggg taacgccagg gtttcccaag tcacgcacgtt 660  
gtaaaacgac ggccagtgaa ttgtataacg actcactata gggcgaattt aaaaaacccc 720  
tcaagacccg ttttagaggcc ccaagggtt atgctagtga attctgcagg gtacccgggg 780  
atcccttaga gatccctcgat cctcgagatc cattgtgtcgcg ggcgggattt tttatcactg 840  
ataagtttgtt ggacatatta tggttatcag tgataaaagt tcaagcatga caaagtgtca 900  
gccgaataca gtgtacccgtg ccggccctgg actgttgaac gaggtcgccg tagacggct 960  
gacgacacgc aaactggcgaa acacggttggg ggtgcagcag cccgcgtttt actggactt 1020  
caggaacaag cggcgctgc tcgacgcact ggccgaagcc atgctggcg agaatcatac 1080  
gcttcgggtc cgagagccga cgacgactgg cgctcatttc tgatcgaa tcccgcagct 1140  
tcaggcaggc gctgtcgcc taccgcgc acaatggatc tcgagggatc ttccatcacct 1200  
accagttctg cgcctgcagg tcgcggccgc gactcttagt acgcgttaagc ttactagcat 1260  
aacccttgg ggcctctaaa cgggtcttga ggggtttttt gagcttctcg ccctatagt 1320  
agtcgttata cagcttgagt attctatagt gtcacctaaa tagcttggcg taatcatgtt 1380  
catagcttgtt tcctgtgtga aattgttatac cgctcacaat tccacacaaac atacgagccg 1440  
gaagcataaa gtgtaaagcc tgggggtcct aatgagttagt ctaactcaca ttaattcg 1500  
tgogctcaact gcccgtttc cagtcgggaa acctgtcggtc cagactgtcat taatgaatcg 1560  
gccaacgcgc ggggagagggc ggttgcgtt ttggcgcgttcc tgcgtcactg 1620  
actcgcgtcg ctcgggtcgat cggctgcggc gagcgttatac agctcactca aaggcggtaa 1680  
tacggttatac cacagaatca ggggataacg cagggaaagaa catgtgagca aaaggccacg 1740  
aaaaggccacg gaacgttaaa aaggcccggt tgctggcggtt tticgtatagg ctccgcaccc 1800  
ctgacgagca tcacaaaaat cgacgctcaa gtcagagggt gcgaaaccccg acaggactat 1860  
aaagataccat ggcgtttccc cctggaaagct ccctcggtcgt ctctctgtt ccgaccctgc 1920  
cgcttacccgg atacctgtcc gcttcttcc cttegggaag cgtggcgct tctcatagct 1980  
cacgctgttag gtatctcgt tgggtgttagg tgggtcgctc caagctgggg tgggtgcacg 2040  
aacccttccgt tcagccgcac cgacttatacg ccaactggcag cagccactgg taacaggatt agcagagcga 2100  
cggtaaagaca ggtatgttagg cgggtctaca ggttcttga agtgggtggcc taactacggc tacactagaa 2160  
ggacagtatt tggtatctgc gcttgcgtatc cggcaaaacaa accaccgtgtt gtacgggtgg tttttttgtt tgcaagcagc 2280  
agattacgcg cagaaaaaaaaa ggtatctcaag aagatcttta gatctttct acggggctg 2400  
acgctcaatc gaaacgaaaac tcacgttaag ggattttggg catgagatca taaaaagga 2460  
tcttcaccta gatcccttta aattaaaaat gaagttttaa atcaatctaa agtatatag 2520  
agtaaactt gtcgtacagt taccatgtc taatcagtga ggcacctatc tcagcgatct 2580  
gtctatttc ttcatccata gttgcctgac tccccgtcgt ttagataact acgataacggg 2640  
agggcttacc atctggcccc cagatttatac agcaataaaac tcttattaaatt gttggggaa agcttagagta agtagtgc 2820  
cagatccgc ctccatccag cagttaatag tttgcgcac cgttggat ggttgcgtt ccacgtcgt 2880  
gggttgcgtt gggttcatc agctccgggtt cccaaacgtc aaggcgagtt acatgatccc 2940  
ccatgttgtc caaaaaaagcg tggccgcagt gttatctactc catccgtaaat atgtttttct ctttgcggg cgtcaataacg ggataatagt gtatgacata 3180  
ggcagaactt aaaagtgtc tcttacccgt gttgagatcc catcttttac ttccatccagc aacgttcttc gggggcaaaaa ctctcaagga 3240  
tcttacccgt gttgagatcc catcttttac ttccatccagc aacgttcttc gggggcaaaaa ctctcaagga 3240  
aaaagggaaat aaggcgaca attgaagcat ttatcagggt aacccactcg tgcacccaaat tgatcttcgt 3300  
aaaataaaaca aatagggtt catccgtaaat ggttctgggt gagaaaaac aggaaggcaa aatgcccac 3360  
aaaccattat tatacatgaca cggaaatgtt gaataactcat actcttccctt tttcaatatt 3420  
tattgtctca tgaacggata catatttgaat tttcaatatt 3480  
ccgcgcacat ttcccccggaa agtgcacccgt gacgtctaaat 3540  
ttaacctataaaaataggcg tatacgtggg cccttcgtc 3600

tcgcgcgttt cggtgatgac ggtaaaaacc tctgacacat gcagctcccg gagacggtca 3660  
cagcttgtct gtaagcggat gccgggagca gacaaggcccg tcagggcgcg tcagcgggtg 3720  
ttggcgggtt tcggggctgg cttaaactatg cggcatcaga gcagattgtt ctga 3774

<210> 10  
<211> 5148  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Plasmid pGN39

<400> 10  
taatacgtact cactataggg cgaattcaaa aaaccctca agaccggtt agaggccccca 60  
aggggttag ctatgtaaatt ctgcagcggt accccggggat cctctagaga tccctcgacc 120  
tcgagatcca ttgtgctgga aagatcacaa gtttacaaa aaaagctgaa cgagaaacgt 180  
aaaatgataat aaatatcaat atattaaatt agatttgca taaaaaacag actacataat 240  
actgtaaaac acaacatatac cagtcaactat ggccggcgca tttaggcaccc caggcttac 300  
actttatgtc tccggctcgat ataatgttg gatTTTgagt taggatccgg cgagattttc 360  
aggagctaa gaagctaaaa tggagaaaaa aatcaactgga tataccaccc ttgatataatc 420  
ccaatggcat cgtaaagaac attttgaggc atttcaacta gttgctcaat gtacctataa 480  
ccagaccgtt cagctggata ttacggccctt tttaaagacc gtaaagaaaaa ataagcacaa 540  
gttttatccg gccttatttc acattcttgc ccgcctgatg aatgctcatac cggaattccg 600  
tatggcaatg aaagacggtg agctggatgt atggatagt gttcaccctt gttacaccgt 660  
tttccatgag caaactgaaa cgatcttcta cacatataatt ccctaaaggg ttatttgaga atatgtttt cgtctcagcc aatccctggg tgagttcac 840  
cagtttgat ttaaacgtgg ccaaatatgga caacttcttcc gcccccggtt tcaccatggg 900  
caaataattat acgcaaggcg acaagggtgt gatgccgctg gcgattcagg ttcatcatgc 960  
cgtctgtat ggcttccatg tcggcagaat gcttaatgaa ttacaacagt actgcgtatga 1020  
gtggcaggc gggcgtaaa gatctggatc cggctacta aaagccagat aacagtatgc 1080  
gtatTTGCGC gctgatTTT gcggtataag aatataact gatatgtata cccgaagtat 1140  
gtcaaaaaga ggtgtgctat gatgtcaata tctccggctt ggttaagcaca accatgcaga 1200  
cagttgtca aggcatataat atgaagcccg tcgtctgcgt gccgaacgct ggaaaacgggaa aatcaggaa gggatggctg 1320  
aggtcgcccc gtttattgaa atgaacggct cttttgtgtc cgagaacagg gactgggtgaa 1380  
atgcagtttta aggtttacac ctataaaaaga gagagccgtt atcgtctgtt tggatgtat 1440  
cagagtata ttattgacac gcccgggoga cggatggtga tccccctggc cagtgcacgt 1500  
ctgtgtcag ataaagtctc ccgtgaacct tacccgggtt tgcataatcg ggtatgaaagc 1560  
tggcgtatg tgaccaccga tatggccagt gtggccgtt ccgttacgg ggaagaagtg 1620  
getgatctca gccaccgcga aatagacatc aaaaacgcca ttaacctgtat tttctgggaa 1680  
atataaaatgt caggctccct tatacacacg cagtcgtcag gtcgaccata gtgactggat 1740  
atgttgtt ttacagtatt atgtgtctg tttttatgc aaaaatctaatt ttaatataatt 1800  
gatatttata tcattttacg agtgcgttcc agtttcttgc tacaagggtt tgatcttcc 1860  
agcacaatgg atctcgaggg cttaaccatcataa cctaccagg tctgcgcctgc aggtcgccgc 1920  
cgcgactcta gacgcgtaa ggggttttt tgagcttctc cttactagca taacccttg gggcctctaa acgggtctt 1980  
aggggttttt tgagcttctc gcccataatg gagtcgtatt acagcttggat tattctatag 2040  
tgtcacctaa atagcttgc gtaatcatgg tcatactgtt ttccctgtgtt aaattgttat 2100  
ccgctcacaa ttccacacaa catacgagcc ggaagcataa agtgtaaagc ctgggggtgcc 2160  
taatgagtgta gctaactcataa attttgcgttcc ggcataatg ggtatcttgc ttgcgttcc 2220  
aacctgtcg tcccgactca ttaatgaatc ggcataacgcg cggggagagg cggtttgcgt 2280  
attggcgctt cttccgttcc ctgcgtcaact gactcgctgc gctcggtcg tccgctgcgg 2340  
cgagcggtat cagctcaactt aaaggcggtt atacgggtt ccacagaatc aggggataac 2400  
gcagggaaaga acatgtgagc aaaaggccag caaaaggcca ggaaccgtaa aaaggcccg 2460  
ttgtggcgat ttttcgtat gctccggcccc cctgacgagc atcacaaaaaa tgcacgtca 2520  
agtcagaggt ggcaaaaccc gacaggacta taaagatacc aggcgtttcc ccctggaaagc 2580  
tccgacccttgc cggcttaccg gataccgtc cgccttctc ttctcatagc tcacgctgtt ggtatctc 2640  
ccttcggaa gctggcgctt ctgtgtgcac gaacccccc ttcagcccgaa ccgtcgcc 2700  
gtcggtcgat ccaagctggg ttatccgtat tgagtcacac ccgttaagac acgacttatac gcaactggca 2760  
ttatccgtat actatcgatc tagcagagcg aggtatgttag gccgtgtcact agagtcttgc 2820  
cgacccactg gtaacaggat

aagtggggc	ctaactacgg	ctacactaga	aggacagttat	tttgttatctg	cgcgttgcgt	2940
aagccagtt	ccttcggaaa	aagagttgg	agctttgtat	ccggcaaaaca	aaccaccgt	3000
ggtagcggt	gttttttgc	tttcaagcag	cagattacgc	gcagaaaaaa	aggatctcaa	3060
gaagatcct	tgtatcccc	tacgggtct	gacgctcagt	ggaacgaaaa	ctcacgtta	3120
gggattttgg	tcatgagatt	atcaaaaagg	atcttcacct	agatcccttt	aaattaaaaa	3180
tgaagttta	aatcaatcta	aagtataatat	gagtaaactt	ggctgacag	ttaccaatgc	3240
ttaatcagt	aggcacctat	ctcagcgtac	tgtctatttc	gttcatccat	agttgcgtga	3300
ctccccgtcg	tgtagataac	tacgataacgg	gagggttac	catctggccc	cagtgcgtca	3360
atgataccgc	gagacccacg	ctcacggct	ccagatttat	cagcaataaa	ccagccagcc	3420
ggaaggccg	agcgcagaag	tggtcctgca	actttatccg	ccctccatcca	gtctattaaat	3480
tgttgccgg	aagctagagt	aagtagtccg	ccagttataa	gttgcgc当地	cgttgtggc	3540
attgctacag	gcatcggt	gtcacgctcg	tcgttggta	tggcttcat	cagctccggt	3600
tcccaacat	caaggcgagt	tacatgatcc	cccatgttgc	gcaaaaaaagc	ggtagctcc	3660
ttcggtcctc	cgatcggt	cagaagtaag	ttggccgc当地	tgttatcact	catggttatg	3720
gcagcactgc	ataattctct	tactgtcatg	ccatccgtaa	gatgctttc	tgtgactggt	3780
gagttactcaa	ccaagtcatt	ctgagaatac	cgccccc当地	gaccgagtt	ctcttgc当地	3840
gcgtcaatac	gggataatag	tgtatgacat	agcagaactt	taaaaagtct	catcatgg	3900
aaacgttctt	cggggcgaaa	actctcaagg	atcttaccgc	tgttgagatc	cagttcgatg	3960
taacccactc	gtgcacccaa	ctgatcttca	gcattttta	cttccaccag	cgttctggg	4020
ttagcaaaaa	caggaaggca	aatatgccca	aaaaaggaa	taagggcgac	acggaaatgt	4080
tgaataactca	tactcttcct	tttcaat	tattgaagca	tttatcaggg	ttattgtctc	4140
atgagcgat	acatatttga	atgtatttag	aaaaataaaac	aaataggggt	tccgcgcaca	4200
tttccccgg	aagtgc当地	tgacgtctaa	gaaaccatta	ttatcatgac	attaacctat	4260
aaaaataggc	gtatcacgag	gcccttctg	ctcgccggt	tcggtgatga	cggtaaaac	4320
ctctgacaca	tgcagctccc	ggagacggc	acagcttgc	tgttaagcgga	tgccgggagc	4380
agacaagccc	gtcaggcgc	gtcagcgggt	gttggcgggt	gtcggggctg	gcttaactat	4440
gcggcattcg	agcagattgt	actgagatg	caccatatgc	ggtgtgaaat	accgcacaga	4500
tgcgttaagga	gaaaataccg	catcaggcga	aattgtaaac	gttaatattt	tgttaaaatt	4560
cgcgttaat	atttggtaaa	tcagcttatt	ttttaaccaa	taggccc当地	tcggcaaaat	4620
cccttataaa	tcaaaaagaat	agaccgagat	agggttgagt	gttggtccag	tttggaaacaa	4680
gagttccacta	ttaaaaagaaacg	tggactccaa	cgtcaaggg	cgaaaaaccg	tctatcaggg	4740
cgtatggccca	ctacgtgaac	catcacccaa	atcaagttt	ttgcggtca	ggcgc当地	4800
agctctaaat	cggaaacccta	aaggggagccc	ccgatttaga	gcttgacggg	gaaagccggc	4860
gaacgtggcg	agaaaaggaag	ggaagaaaagc	gaaaggagcg	ggcgctaggg	cgctggcaag	4920
tgttagcggtc	acgctcgccg	taaccaccac	acccggccgc	cttaatgcgc	cgctacaggg	4980
cgcgtccatt	cgccattcag	gctgcgc当地	tgttggaaag	ggcgatcggt	gcgggc当地	5040
tcgcttattac	gccagctggc	gaaaggggg	tgtgtc当地	ggcgattaa	ttgggtaacg	5100
ccagggtttt	cccagtcaag	acggtgtaaa	acgacggcc	gtgaattt		5148

```
<210> 11
<211> 3715
<212> DNA
<213> Artificial Sequence
```

<220>  
<223> Description of Artificial Sequence: Plasmid  
TopoRNAi

```

<400> 11
gagtgcacca tatgcgttgc gaaataccgc acagatgcgt aaggagaaaa taccgcattca 60
ggcggaaattt taaaacgttaa tattttgtta aaatttcgcgt taaatatttg ttaaatcagc 120
tcatttttta accaataggc cgaaatcgcc aaaatccctt ataaatcaaa agaatacgacc 180
gagatagggt tgagtgttgt tccagtttgg aacaagagtc cactattaaa gaacgtggac 240
tccaacgtca aaggcgaaaa aaccgtctat cagggcgatg gccccactacg tgaaccatca 300
cccaaatcaa gtttttgcg gtcgaggtgc cgtaaagctc taaatcgaa ccctaaagggg 360
agcccccgat ttagagcttg acggggaaag ccggcgaacg tggcgagaaa ggaagggaag 420
aaagcgaaaa gagcgggcgc tagggcgctg gcaagtgtag cggtcacgt gcgcgttaacc 480
accacacccg cccgcgttaa tgccgcgtta cagggcgctg ccattcgcca ttcaaggctgc 540
gcaactgttg ggaaggcgaa tcggtgccgg cctttcgctt attacgcccag ctggcgaaag 600
ggggatgtgc tgcaaggcgaa ttaagttggg taacgccagg gttttcccaag tcacgacgtt 660
gtaaaacgcac ggccagtggaa ttgtataatcg actcaactata gggcgaattc aaaaaacccc 720

```

<210> 12

<211> 4107

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Plasmid pGN49A

<400> 12

tgtataacgatctactatagggcgaattcaaaaaacccttcaagaccgttttagaggccc 60

caagggttta tgctagtgaa ttctgcagcg gtaccgggg atccctctaga gatccctcg 120  
cctcgagatc catttgctg gaaaggatct ggatccgct tactaaaagc cagataacag 180  
tatgcgtatt tgccgcgtg ttttgcgt ataagaatat atactgatat gtatacccg 240  
agtatgtcaa aaagagggtgt gctatgaagc agcgattac agtgacagg gacagcgaca 300  
gctatcagtt gctcaaggca tatatgtatg caatatctcc ggtctggtaa gcacaaccat 360  
gcagaatgaa gcccgtcg tcgtgccg acgctggaa gcggaaaatc aggaaggat 420  
ggctgagggtc gcccgggta ttgaaatgaa cggtcttt gctgacgaga acaggactg 480  
gtgaaatgca gtttaagggt tacacctata aaagagagag ccgttatcgt ctgttgtg 540  
atgtacagag tgatattatt gacacgcccc ggcgacggat ggtgatcccc ctggccagtg 600  
cacgtctctt aagcgataaa gtctcccgta aacttaccc ggtgggtcat atcggggat 660  
aaagctggcg catgatgacc accgatatgg ccagtgcc ggtctccgtt atcggggaaag 720  
aagtggctga tctcagccac cgcgaaaatg acataaaaaa cgccattaaac ctgatgttct 780  
gggaaatata aatgtcaggc tcccttatac acagccttc cagcacaatg gatctcgagg 840  
gatctccat acctaccagt tctgcgcctg caggtcgccg ccgcgactct agacgcgtaa 900  
gttacttagc ataaccctt ggggcctta aacgggtctt gaggggttt ttgagcttct 960  
cgccctatacg tgagtcttatc tacagctga gtattctata gtgtcaccta aatagcttgg 1020  
cgtaatcatg gtcatagctg tttcctgtgt gaaattgtta tccgctcaca attccacaca 1080  
acatacgagc cggaagcata aagtgtaaag cctgggggtgc ctaatgagtg agctaactca 1140  
cattaattgc gttgcgcctca ctgcccgtt tccagtcggg aaacctgtcg tgccagctgc 1200  
attaatgaat cgcccaacgc gccccggagag gcggtttgcg tattggcgc tcttccgctt 1260  
cctcgctcac tgactcgctg cgctcggtcg ttccggctcg gcgagcggtg tcagctcact 1320  
caaaggcggt aatacggtt tccacagaat caggggatata cgccagggaaag aacatgtgag 1380  
caaaaggcca gcaaaaggcc aggaaccgta aaaaggccgc gttgctggcg ttttcgata 1440  
ggctccgccc ccctgacgag catcacaaaa atcgacgctc aagttagcagg tggcgaaacc 1500  
cgacagact aaaaagatac caggcggttc cccctggaaag ctccctcg cgctctccgt 1560  
ttccgaccct gcocttacc ggataccctg ccgccttct cccttcggga agcgtggcgc 1620  
tttctcatag ctoacgctgt aggtatctca gttcgggtga ggtcggtcgc tccaagctgg 1680  
gctgtgtgca cgaaccccccc gttcagcccg accgctgcgc cttatccgt aactatcg 1740  
ttgagttccaa cccggtaaga cacgacttat cgccactggc agcagccact ggtaacagga 1800  
tttagcagagc gaggtatgta ggccgtgtc cagagtctt gaagtgggtt cctaactacg 1860  
gctacactag aaggacagta ttggtatct ggcgtctgt gaagccagg accttcggaa 1920  
aaagagttgg tagtcttgc tccggcaaac aaaccaccgc tggtagcgtt ggtttttttg 1980  
tttgcagca gcaagattacg cgcagaaaaa aaggatctca agaagatctt ttgatctttt 2040  
ctacgggtc tgacgctcag tggAACggaaa actcacgtt aaggattttt gtcatgagat 2100  
tatcaaaaag gatcttcacc tagatccccc taaattaaaa atgaagttt aatcaatct 2160  
aaagtatata tgatgaaact tggctgaca gttaccaatg cttaatcgt gaggcaccta 2220  
tctcagcgat ctgtcttattt cgttcatcca tagttgcctg actccccgtc gtgtagataa 2280  
ctacgatacg ggagggctta ccatctggcc ccagtgcgtc aatgataaccg cgagaccac 2340  
gctcacccgc tccagattt tcagcaataa accagccagc cggaaaggccc gagccgagaa 2400  
gtggctctgc aactttatcc gctccatcc agtctattaa ttgttgcggg gaagctagag 2460  
taagtagttc gccagttat agtttgcgca acgttgggtt cattgtaca ggcacgtgg 2520  
tgtcacgctc gtgttttgtt atggcttcat tcagctccgg ttcccaacga tcaaggcgag 2580  
ttacatgatc ccccatgttg tgcaaaaaag cggttagctc cttcggctt ccgatcggtt 2640  
tcagaagtaa gttggccgca gtgttacac tcatggttat ggcagcactg cataattctc 2700  
ttactgtcat gccatccgta agatgcttt ctgtgactgg tgtagtactca accaagtcat 2760  
tctgagaata ccgegccccg cgaccggatt gctcttgcctt ggcgtcaata cgggataata 2820  
gtgtatgaca tagcagaact taaaagtgc tcatcattgg aaaacgttct tcggggcgaa 2880  
aactctcaag gatcttaccg ctgttggat ccagttcgat gtaacccact cgtgcaccca 2940  
actgatcttc agcatctttt actttcacca gcgttctgg gtcgttccatc acggaaaggc 3000  
aaaatgccgc aaaaaaggga ataagggcga cacggaaaatg ttgaataactc atactcttcc 3060  
ttttcaata ttattgaagc atttatcagg ttattgtct catgagcgga tacatatttgc 3120  
aatgtatata gaaaaataaa caaatagggg ttccgcgcac atttccccca aagtgcac 3180  
ctgacgtcta agaaaaccatt attatcatga cattaaccta taaaatagg cgtatcacga 3240  
ggccctttcg tctcgccgt ttccggatg acgtggaaa cctctgacac atgcagctcc 3300  
cgagacggc cacagcttgt ctgtaaagcgg atgcccggag cagacaagcc cgtcaggccg 3360  
cgtcagccgg tggccgggg tggccggct ggcttaacta tgccgttccatc gagcagattg 3420  
tactgagagt gcaccatatg cgggtgtaaa taccgcacag atgcgttccatc agaaaatacc 3480  
gcatcaggcg aaattgtaaa ctgttataatt ttgttaaaat tcgcgtttaa tattttttaa 3540  
atcagctcat ttttttacca ataggccgaa atcggcaaaa tcccttataa atcaaagaa 3600  
tagaccgaga taggggtttag tggccatca gtttggaaaca agagtccact attaaagaac 3660  
gtggactcca acgtcaaaagg qcgaaaaacc gtctatcagg gcgatggccc actacgtgaa 3720

ccatcaccca aatcaagttt tttgcggctcg aggtgccgta aagctctaaa tcggaaacct 3780  
 aaaggagcc cccgatttag agcttgacgg ggaaagccgg cgaacgtggc gagaaaggaa 3840  
 gggaaagaaag cgaaaggagc gggcgctagg gcgcgtggcaa gtgtagcggt cacgcgtgcgc 3900  
 gtaaccacca caccgcgcg gcttaatgcg ccgctacagg ggcgtccat tcgccattca 3960  
 ggctgcgca ctgttggaa gggcgatcg tgccggctc ttgcgttatta cgccagctgg 4020  
 cgaagggggg atgtgtcga aggcgattaa gtgggtaac gccagggttt tcccagtac 4080  
 gacgttgtaa aacgacggcc agtgaat 4107

&lt;210&gt; 13

&lt;211&gt; 4001

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Plasmid pGN59A

&lt;400&gt; 13

gagtgcacca tatgcggtgt gaaataccgc acagatgcgt aaggagaaaa taccgcata 60  
 ggcgaaattt taaacgttaa tattttgtta aaattcgcgt taaatatttg ttaaatcagc 120  
 tcattttta accaataggc cgaaatcgc 300  
 gagatagggc tgagtgttgc tccagtttgg aacaagagtc cactattaaa gaacgtggac 240  
 tccaaacgtca aaggcgaaa aaccgtctat cagggcgatg gcccaactacg tgaaccatca 300  
 cccaaatcaa gtttttgcg gtcgagggtc cgtaaagctc taaatcgaa ccctaaggg 360  
 agcccccgat ttagagcttgc acggggaaag cccgcaacg tggcgagaaa ggaagggaaag 420  
 aaagcgaaag gacggggcgc tagggcgctg gcaagtgttag cggtcacgct ggcgttaacc 480  
 accacacccg cccgcgtttaa tgcgcgteta cagggcgatg ccattcgcca ttcaaggctgc 540  
 gcaactgttgc ggaaggcgca tgggtgcggg cctctcgct attacgcacg ctggcgaaag 600  
 ggggatgtgc tgcaaggcgca ttaagtggg taacgcagg gttttcccg tcacgcacgtt 660  
 gtaaaacgac gcccagtgaa ttgtataacg actcactata gggcgaattc gagctcggt 720  
 cccggggatc ctctagagat ccctcgacat cgagatccat ttgtctggaa aggtatcgaa 780  
 tccggcttac taaaagccag ataacagttat gctgttgc gogctgattt ttgcgttata 840  
 agaatatata ctgtatgttataccgaat atgtcaaaaaa gaggtgtgtc atgaagcagc 900  
 gtattacagt gacagttgc acgcacagat atcagttgtc caaggcatat atgatgtcaa 960  
 tatctccggc ctggtaagca caaccatgca gaatgaagcc cgtcgctgc gtggcaacg 1020  
 ctggaaagcg gaaaatcagg aagggtgc tgaggcgcc cgggttattt aatgaacgg 1080  
 ctcttttgcg tgcgagaaca gggactggg aatgcagttt taagggttac acctataaaaa 1140  
 gagagagccg ttatcgctg ttgtggatg tacagagtta tattattgac acgccccggc 1200  
 gacggatggt gatccccctg gccagtgcac gtctcttaa cgataaagtc tcccgtaac 1260  
 tttaccgggt ggtgcataatc ggggatgaaa gctggcgcat gatgaccacc gatatggca 1320  
 gtgtgcgggt ctccgttatac ggggaaagat tggctgtatc cagccaccgc gaaaatgaca 1380  
 tcaaaaacgc cattaaacctg atgttctgg gaatataat gtcaggctcc cttatacaca 1440  
 gcctttcccg cacaatggat ctcgaggat cttccataacc taccagttct ggcgcgtc 1500  
 gtcgcggccg cgactctcta gactcgaaat cttctcgccc tataatgtgatc cgtattacag 1560  
 cttgatgttattt ctatgtgtc acctaaatag cttggcgtaa tcatggtcat agctgttcc 1620  
 tgggtgaaat tggatccgc tcacaattcc acacaacata cgagccggaa gcataaagt 1680  
 taaagcttgg ggtgcataat gactcgatc actcacatta attgcgttgc gtcactgcc 1740  
 cgctttcccg tcggaaacc tgcgtgcac gctgcattaa tgaatcgcc aacgcgcggg 1800  
 gagaggcggt ttgcgttattt ggcgtcttc cgcttcctcg ctcactgtact cgctgcgtc 1860  
 ggtcggttccg ctggcgccgat cggtatcgc tcactcaatc ggcgtataatc ggttattccac 1920  
 agaatcaggc gataacgcac gaaagaacat gtgagcaaaa ggcgcggcaaa aggcaggaa 1980  
 ccgtaaaaag gccgcgttgc tggcggttcc cgtatggctc cgccccccctg acgagcatca 2040  
 caaaaatcgca cgctcaagtc agagggtggc aaaccgcaca ggactataaa gataccaggc 2100  
 gtttccccc ggaagctccc tcgtgcgtc tccgttccg accctgcgcg ttaccggata 2160  
 cctgtccggc ttctccctt cgggaagcgt ggcgtttct catagctcac gctgttaggt 2220  
 tctcgttgc gttgttaggtcg ttgcgtccaa gctggcgatc gtgcacgaaac cccccgttca 2280  
 gccccgaccgc tgcgccttat cggtaacta tcgttgcgt tccaaaccgg taagacacga 2340  
 cttatcgcca ctggcagcag ccactggtaa caggattagc agagcgaggt atgtaggcg 2400  
 tgctacagat ttcttgcgtt ggtggctaa ctacggctac actagaagga cagtatttgg 2460  
 tatctgcgt ctgtgtcaatc cgttacattt cggaaaaaga gttggtagct tttgatccgg 2520  
 caaacaacc accgctggta gcggtggtt ttttgcgttgc aagcagcaga ttacgcgcag 2580  
 aaaaaaaaaa gtcagaagat atcctttgtat ctttctacg gggctgtacg ctcagtggaa 2640

cgaaaactca cguttaaggga ttttggcat gagattatca aaaaggatct tcacctagat 2700  
 ccttttaat taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc 2760  
 tgacagttac caatgtttaa tcagtgggc acctatctca gcgatctgtc tatttcgttc 2820  
 atccatàgtt gcctgactcc cctgtgtta gataactacg atacgggagg gcttaccatc 2880  
 tggcccagt gctgcaatga taccgcgaga cccacgctca cccgctccag atttatcagc 2940  
 aataaaccag ccagccggaa gggccgagcg cagaagtgg ctgcgaactt tatccgcctc 3000  
 catccagttt attaattgtt gccgggaagc tagagaagt agttcgccag ttaatagttt 3060  
 gcgcaacgtt gttggcattt ctacaggcat cgtgggtca cgctcgctgt ttggatggc 3120  
 tticattcagc tccggttccc aacgatcaag gcgaggtaa tgatccccca tgggtgtcaa 3180  
 aaaagcggtt agctccctcg gtccctccgt cgttgcaga agtaagtgg ccgcagtgtt 3240  
 atcactcatg gttatggcag cactgcataa ttcttctact gtcatgccat ccgtaagatg 3300  
 cttttctgtg actggtgagt actcaaccaa gtcattctga gaataccgcg cccggcgacc 3360  
 gagttgcctt tgccggcgt caatacggta taatagtgtt tgacatagca gaactttaaa 3420  
 agtgctcattt attgaaaac gttcttcggg gcgaaaactc tcaaggatct taccgcgttt 3480  
 gagatccagt tcgatgtaac ccactcggtc acccaactga ttttcagcat cttttacttt 3540  
 caccagcggtt tctgggtgag caaaaacagg aaggccaaaat gccgcaaaaa aggaaataag 3600  
 ggcgacacgg aaatgttta tactcataact cttccctttt caatattattt gaagcattha 3660  
 tcagggttat tgtctcatga gcgatcacat atttgaatgt atttagaaaa ataaaacaaat 3720  
 aggggttccg cgacatttc cccgaaaagt gccacctgac gtcataagaaa ccattattat 3780  
 catgacattha acctataaaa ataggcgat cacgaggccc tttcgctctcg cgcgttccg 3840  
 tggatgcgtt gaaaacctct gacacatgca gtcggggag acggtcacag cttgtctgtt 3900  
 agcggatgcc gggagcagac aagccgtca gggcgcgtca ggggtgttg ggggtgtcg 3960  
 gggctggctt aactatgcgg catcagagca gattgtactg a 4001

&lt;210&gt; 14

&lt;211&gt; 36

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:  
Oligonucleotide oGN103

&lt;400&gt; 14

taccaaggct agcatggttt atcactgata agttgg

36

&lt;210&gt; 15

&lt;211&gt; 34

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:  
Oligonucleotide oGN104

&lt;400&gt; 15

taccaaggct agcatgggcc tgcctgaagg ctgc

34

&lt;210&gt; 16

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:  
Oligonucleotide oGN126

&lt;400&gt; 16

gatctggatc cggcttacta aaagccagat aacagtatgc

40

&lt;210&gt; 17

<211> 46  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide oGN127

<400> 17  
ggagacttta tcgcttaaga gacgtgcact ggccaggggg atcacc 46

<210> 18  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide oGN128

<400> 18  
ccagtgcacg tctcttaagc gataaagtct cccgtgaact ttacccggtg g 51

<210> 19  
<211> 37  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide oGN129

<400> 19  
gctgtgtata agggaggctg acatttatat tccccag 37

<210> 20  
<211> 375  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR fragment  
generated by primers oGN103 and OGN104 on pCDM8

<400> 20  
taccaaggct agcatggttt atcactgata agttggataa gttgggtggac atattatgtt 60  
tatcagtgtat aaagtgtcaa gcatgacaaa gttcagccg aatacagtga tccgtgccgg 120  
ccctggactg ttgaacgagg tcggcgtaga cggctctgacg acacgcaaac tggcgaaacg 180  
gttgggggtt cagcagccgg cgcttactg gcacttcagg aacaagcggg cgctgctcga 240  
cgcaactggcc gaagccatgc tggcggagaa tcatacgctt cggtgccgag agccgacgac 300  
gactggcgct catttctgtat cgggaatccc gcagcttcag gcaggccat gctagccttg 360  
gtaccagcac aatgg 375

<210> 21  
<211> 670  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR fragment

&lt;400&gt; 21

gatctggatc cggcttacta aaagccagat aacagtatgc gtatttgcgc gctgattttt 60  
gcggtaataag aatatatact gatatgtata cccgaagtat gtcaaaaaga ggtgtgctat 120  
gaagcagcgt attacagtga cagttgacag cgacagctat cagttgctca aggcatatat 180  
gatgtcaata tctccggctc ggttaaggcaca accatgcaga atgaagcccg tcgtctgcgt 240  
gccgaacgct ggaaagcggaa aatcaggaa gggatggctg aggtcgcccg gtttattgaa 300  
atgaacggct ctttgctga cgagaacagg gactggtcaa atgcagtttta aggtttacac 360  
ctataaaaga gagagccgtt atcgtctgtt tgtggatgtt cagagtgtata ttattgacac 420  
gcccgggcga cggatggtga tccccctggc cagtgcacgt ctcttaagcg ataaagtctc 480  
ccgtgaacctt taccgggtgg tgcatatcg ggatgaaagc tggcgcatga tgaccaccga 540  
tatggccagt gtgcggctc cggatggtgg ggaagaagtg gctgatctca gccaccgcga 600  
aatgacatc aaaaacgcctttaacctgtat gttctgggaa atataaatgt caggctccct 660  
tatacacacgc 670

## INTERNATIONAL SEARCH REPORT

ntional Application No  
PCT/IB 01/01068

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 7 C12N15/10 C12N15/63 C12N15/70 C12N1/21		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 7 C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the International search (name of data base and, where practical, search terms used)		
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, MEDLINE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 782 325 A (PROTEUS) 18 February 2000 (2000-02-18) page 7, line 20 -page 8, line 8 page 11, line 11 - line 36 page 23, line 31 -page 24, line 9 ---	1-24, 26, 27
A	WO 00 01846 A (DEVGEM N.V.) 13 January 2000 (2000-01-13) cited in the application page 8, line 9 -page 10, line 22 page 15, line 9 - line 33 page 21, line 21 -page 22, line 29 ---	1-28
	-/-	
<input checked="" type="checkbox"/>	Further documents are listed in the continuation of box C.	<input checked="" type="checkbox"/> Patent family members are listed in annex.
* Special categories of cited documents :		
'A' document defining the general state of the art which is not considered to be of particular relevance		
'E' earlier document but published on or after the international filing date		
'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
'O' document referring to an oral disclosure, use, exhibition or other means		
'P' document published prior to the international filing date but later than the priority date claimed		
'T' later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention		
'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone		
'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.		
'&' document member of the same patent family		
Date of the actual completion of the international search	Date of mailing of the international search report	
20 September 2001	27/09/2001	
Name and mailing address of the ISA	Authorized officer	
European Patent Office, P.B. 5818 Palentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B	

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 01/01068

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 34815 A (CAMBRIA BIOSCIENCES, LLC) 17 May 2001 (2001-05-17) page 5, last paragraph -page 6, paragraph 4 page 20, paragraph 2 page 24, last paragraph; example 1 page 13, last paragraph -page 15, paragraph 2 -----	1-10,12, 22,23,25

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

ational Application No

PCT/IB 01/01068

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
FR 2782325	A	18-02-2000		FR 2782325 A1 AU 5171699 A BR 9912934 A EP 1104489 A1 WO 0009747 A1		18-02-2000 06-03-2000 08-05-2001 06-06-2001 24-02-2000
WO 0001846	A	13-01-2000		AU 4907999 A WO 0001846 A2 EP 1093526 A2 GB 2349885 A NO 20010019 A		24-01-2000 13-01-2000 25-04-2001 15-11-2000 05-03-2001
WO 0134815	A	17-05-2001		AU 1461701 A WO 0134815 A1		06-06-2001 17-05-2001